Volume LXXIII Number 1

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

JANUARY 1922

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The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

37
1922

Volume LXXIII Number 1

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued January 18, 1922

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Bolivia, Colombia, Honduras, Nicaragua, Peru, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 35 cents on annual subscriptions (total $9.35), on single copies, 3 cents (total 78 cents); for all other countries in the Postal Union, 84 cents on annual subscriptions (total $9.84), on single copies, 11 cents (total 86 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, 64*s.* each; single copies, including postage, 5*s.* 6*d.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 19.75 each; single copies, including postage, Yen 1.75 each.

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Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased.

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Separates, if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proofs; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

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VOLUME LXXIII NUMBER 1

THE
BOTANICAL GAZETTE

January 1922

NONSYMBIOTIC GERMINATION OF ORCHID SEEDS

LEWIS KNUDSON

(WITH THREE FIGURES)

The germination of orchid seeds for a long time has been recognized as difficult and generally uncertain of attainment. Practical orchid growers for years have attempted to find a method which will insure germination. They meet with success at times, but fail utterly on a second attempt with the same method. Moreover, two sowings made at the same time and under apparently identical conditions may result in germination in the one case and failure in the other. There are growers in England, France, and also the United States who, if one may believe reports, are consistently successful in germinating the seeds of the commercially important orchids. The grower, however, is naturally unwilling to part with the details of his method. From the scientific aspect it is doubtful whether he can explain the cause of his success. Generally speaking it may be stated that practical orchid growers have not yet solved the problem of producing orchid plants from seeds.

The difficulty of germinating seeds of orchids is due in part to inherent causes, but undoubtedly is due also to environmental factors. The extremely small size of the embryo renders it liable to death if it becomes desiccated. Generally the seeds are sown on a substratum rich in organic matter, such as sawdust, leaf mold, wood or bark, peat, sphagnum, or mixtures of the two last-named substances. These substances are favorable for the growth

of fungi and algae, and the embryos may be killed because of being covered by these organisms, or more likely by injurious substances produced by the decomposition of these organisms. The work of BURGEFF (4) and BERNARD (2) demonstrates that death may be due to pathogenic fungi, and the writer's experiments in transplanting seedlings from tubes to open pots demonstrate clearly this danger. In addition to these factors, attention must be given to preventing loss due to insect pests. As suggested, however, there are apparently inherent characteristics of the seeds which make for refractory germination. It is this which attracted the attention of BERNARD, who in a number of publications presented evidence tending to show that the germination of the seeds and the subsequent growth of the seedlings are dependent upon infection by certain strains of the fungus which generally is found living in the orchid root, and which BERNARD considered to be *Rhizoctonia*. BURGEFF came to substantially the same conclusions, maintaining that germination was possible only when the embryo became infected with the proper strain of the fungus, to which he gave the name *Orcheomyces*, without attempting to classify it.

BERNARD and BURGEFF both pointed out that infection of the embryo began at the suspensor end of the seed, and that in the case of *Cattleya* and related forms the primary infection occurred through the delicate suspensor. Growth occurred if only the lower portion of the embryo became infected, and if the infection continued beyond approximately the lower third of the embryo, then death of the embryo resulted. It was also observed in germinating embryos that the fungus disintegrated in the infected zone, forming clumps of disintegrated hyphal material in the cells similar to the clumps found in cells of the root. It was the opinion of BERNARD that the fungus was digested by the orchid embryo. The essential point to be noted, however, is that a delicate balance between the host and the fungus apparently must be maintained in order to insure germination and also to prevent death of the embryo.

Granting for the present that a symbiotic relationship exists between the fungus and the embryo, it is nevertheless true that failure of germination is more common than success, even when the fungus is provided. BERNARD's experiments reveal case after

case in which the introduction of the fungus was followed by death of the seeds or failure to germinate. He states as follows:

> The germination by inoculation is not obtained without certain difficulties. For five years I have sown seeds of diverse species of orchids in culture tubes, each of which contained 100 seeds, and these I have inoculated with *Rhizoctonia* obtained from the roots. Altogether, I have obtained a few hundreds of seedlings, but I underestimate when I place the number of seeds used in my experiments at 50,000. For the majority of the seeds, the association with the fungus that I have placed in their presence has been merely passive and without effect, or impossible or rapidly injurious to the embryos.

The explanation generally offered in these cases is that "activity" of the fungus was altered or the proper strain was not employed, so that the essentially delicate balance between the fungus and the embryo was not maintained.

In certain experiments BERNARD succeeded in germinating seeds of *Cattleya* and *Laelia* without the intervention of the fungus. This was accomplished by using a more concentrated solution of salep. Salep (KING, **6**) is the dry powder obtained by pulverizing tubers of certain orchids, and contains, principally, mucilage 48 per cent, starch 27 per cent, and proteins 5 per cent. It probably contains also some sugar as well as soluble mineral matter. The seedlings obtained in this way were in every respect normal and the germination was very regular. BERNARD suggests that some such method might be developed for practical purposes, since the results with the fungus are so unsatisfactory.

The increasing importance of orchid culture in America, the difficulties in and the restrictions on the importation of orchid plants, and the desirability of creating new hybrid forms, make particularly desirable a method for germinating the seeds. Certain data from the experiments of BERNARD and BURGEFF, indicating that soluble organic compounds might cause germination, and my own previous experiments (**7**) on the organic nutrition of plants, demonstrating that various sugars have a very favorable influence on growth, are indications that germination of orchid seeds might be obtained by the use of certain sugars. This proved to be true.

The results here reported describe a method for germinating the seeds under sterile conditions, the influence of certain sugars

on growth of the embryos, the influence of different concentrations of sugar on growth, the effects induced by certain plant extracts, the favorable influence of certain bacteria, and experiments on transplanting. In the discussion are treated critically the ideas expressed by BERNARD and BURGEFF with respect to the function of the fungus.

For a clearer understanding of the data that follow, it is desirable to trace briefly the mode of development in the germination of seeds of *Cattleya* and *Laelia*. For a detailed discussion, BERNARD'S paper should be consulted. The embryo is somewhat oval-shaped, and is undifferentiated except that the cells at the basal region are large, while those at the apical region are smaller. This is the meristematic region. At the base is subtended a delicate suspensor. The embryo is inclosed within a transparent integument with an opening at the lower end through which the suspensor may protrude. The maximum length of the embryo of *Cattleya* or *Laelia* is about 250μ and the width about 75μ.

Germination consists, first, in an enlargement of the embryo in a transverse direction until a small spherule stage is reached. Accompanying this development there is the formation of chlorophyll, generally more pronounced in the meristem region. The embryo when it ruptures the integument has a width of about 175μ and a length of about 270μ. At the time of rupturing the integument, absorbing hairs begin to grow out from the epidermis. Subsequent development consists in a further enlargement of the embryo, other absorbing hairs begin to develop near the basal region, and there is attained a large spherule or top-shaped structure characterized by a marked depression at the upper surface. Following this there appears in the middle of the depression the first leaf point, which subsequently develops into the first leaf. During this period there is a continued increase in the diameter of the embryo, so that a disklike structure is formed which has been termed by BERNARD the protocorm. At the meristematic region a second and a third leaf may unfold, elongation may occur, and a distinct stem is apparent. The first root may arise either from the protocorm or from the stem below the second or the third leaf. The period required for these developments is generally

from four to six months. Under greenhouse conditions this advanced stage has apparently been attained in some cases in a shorter time.

Methods

Unless otherwise indicated, all cultures were made using agar slopes in culture tubes 180 mm. × 18 mm. The nutrient solution used was either Pfeffer's or a modification referred to hereafter as solution B. The solutions were made up as follows:

Solution B	Pfeffer's
$Ca(NO_3)_2$, 1 gm.	$Ca(NO_3)_2$, 4 gm.
K_2HPO_4, 0.25 gm.	K_2HPO_4, 1 gm.
$MgSO_47H_2O$, 0.25 gm.	$MgSO_47H_2O$, 1 gm.
$Fe_2(PO_4)_3$, 0.05 gm.	KNO_3, 1 gm.
$(NH_4)_2SO_4$, 0.50 gm.	KCl, 0.5 gm.
Distilled H_2O, 1 l.	$FeCl_3$, 40 mgm.
	Distilled H_2O, 5 l.

Solution B was used because Burgeff stated that the orchid seeds utilized ammonium sulphate to better advantage than the nitrate salt. My own experience is not in accordance with this.

Generally 1.50 per cent agar was used, and all media and vessels were autoclaved at fifteen pounds pressure for thirty minutes. To prevent the lodging of spores and microorganisms on the cotton stopper of the culture tube, it was capped with a small vial which, fitting tightly over the cotton plug, inclosed the upper third of the tube. The use of the vial cap was essential because otherwise, under the moist greenhouse conditions, contamination resulted from spores growing down through the cotton plug or between the plug and the tube. By using the vial cap cultures remained pure even after a year in the greenhouse.

The cultures were all grown under aseptic conditions. For sterilizing the seeds, the calcium hypochlorite method of Wilson (13) was used. For this purpose 10 gm. of calcium hypochlorite was added to 140 cc. of distilled water. This was vigorously shaken for a few minutes and then filtered. The clear filtrate was used for sterilizing the seeds. The quantity of seeds desired was placed in a small test tube and the clear filtrate added. The tube was then shaken until each seed became moistened with the solution.

This was repeated several times, since the seeds generally float together in a mass at the surface of the liquid. The period of exposure was about fifteen minutes, although in some preliminary experiments with seeds of *Cattleya* and *Laelia* no injury was noted after a three hours' exposure. The seeds were transferred from the sterilizing solution, without any previous rinsing in water, by the use of a platinum needle. With the small loop used, it was possible to pick up about 100 seeds. These were scattered over the surface of the agar slope. The cultures were maintained in moist chambers in the greenhouse shaded by cheesecloth from direct sunlight, with the temperature between 15° and 35° C. In determining growth, the embryos were measured by means of an ocular micrometer. As was shown by both BERNARD and BURGEFF, the width of the embryo or the protocorm may be accepted as a good criterion of the degree of growth. Other data are included, such as percentage of germination, time of formation of first leaf, color, starch content, etc.

Preliminary experiments

EXPERIMENT 1.—On December 7, 1918, seeds of *Cattleya Schroederae*×*C. gigas* were sterilized by treating them for two hours with the calcium hypochlorite solution. The seeds were sown on agar slopes. The medium used in one case was an extract of peat, made by autoclaving 300 gm. of bog peat, such as is used for potting orchids, with 1200 cc. of tap water. This was filtered and the clear brownish filtrate used. The other medium was made by autoclaving 400 gm. of dormant canna tubers with 600 cc. of water for thirty minutes. By January 7, 1918, the seeds on both were in the small spherule stage and were green. On April 10, four months after planting, the seeds on the canna medium had germinated, the seedlings having one and two leaves. On the peat agar medium the embryos were a little larger than on January 7, 1919, but not significantly different.

EXPERIMENT 2.—The media used were extracts of carrot and garden beet. The carrot extract was made by autoclaving 70 gm. of young carrots (root) with 75 cc. of tap water, and the garden beet extract was made by autoclaving 50 gm. of young beets (root)

with 75 cc. of water. The extracts were filtered, and to the clear filtrate 1.25 per cent agar was added. Seeds of *Cattleya labiata* × *C. aurea* were sterilized and planted on February 14, 1919. On May 13 some of the seeds in each had germinated and the remainder were almost germinated, that is, they were just at the point of producing the first leaf.

EXPERIMENT 3.—The media used were Pfeffer's alone and Pfeffer's plus 1 per cent sucrose. Seeds of *Cattleya mossiae* were planted on January 14, 1919. On July 1 the seeds in the sucrose culture had germinated, one leaf showing. On the Pfeffer's alone the embryos were in a small green spherule stage, the diameter being about 250μ, while the diameter of the embryos on sucrose was about 1000μ.

EXPERIMENT 4.—Seeds of *Cattleya intermedia* × *C. Lawrenceana* were sown on July 18, 1919, on solution B plus 2 per cent glucose on the one hand and 2 per cent sucrose on the other. Owing to an absence from the University, the cultures were not examined until June 9, 1920. At that time, in both glucose and sucrose cultures, the seedlings were well developed, although the culture media had lost most of the water by evaporation. The seedlings had two or three leaves and one or two roots, some of the roots being 4 mm. in length.

Influence of certain sugars and plant extracts on germination

The preliminary experiments show that germination of seeds of *Cattleya* and *Laelia* is possible without the aid of the fungus, provided soluble organic substances are present, particularly sugars. In all these cases the leaf point appeared only after three months, and yet under practical greenhouse conditions, when the seeds are sown merely on a compost of peat and sphagnum or other organic material, the leaf points may appear in a shorter time. For example, according to Mr. T. L. MEAD, of Oviedo, Florida, seeds of *Cattleya* have shown leaf points in as short a period as thirty-five days. Some of the media used by him were oak bark, magnolia bark, and a compost of decayed leaves and sphagnum.

It is possible, of course, that under these practical conditions the fungus is a factor in the growth. Is it possible also that certain

of the products produced on decomposition of the organic substances, such as the auximones described by BOTTOMLEY (3) or the vitamine water-soluble B, are involved in the germination of orchid seeds? Of course other factors may be involved, such as the hydrogen ion concentration, mineral salts, or the rate of transpiration, particularly as influencing the organic composition of the plant.

That a full nutrient medium plus sugar is not capable of sustaining continued growth of higher plants was shown by KNUDSON and LINDSTROM (8) in their experiments with albino corn. The plants kept either in the light or in the dark and supplied with one of several different sugars all died after a month or two. These experiments, together with the work of BOTTOMLEY on auximones, the beneficial influence of vegetable extracts on the growth of fungi recently described by DUGGAR (5) and by WILLAMAN (11), and the beneficial influence of vegetable extracts on the growth of yeast as described by WILLIAMS (12) and by BACHMANN (1) suggest that more rapid germination and more vigorous plants could be obtained if a vegetable extract was added to the nutrient medium.

With no idea of determining what specific substances are involved in stimulating growth, but in the endeavor to develop a rapid and effective method for the germination of seeds of certain orchids, the experiments described in table I were made. The nutrient solution used was solution B.

The extracts used were prepared as follows. Potato extract: 200 gm. of new potato with the skin removed, with 300 cc. of distilled water; wheat extract: 200 gm. of air-dried soft wheat, with 300 cc. of distilled water; beet extract: 200 gm. of a red garden beet cut into small pieces, with 200 cc. of distilled water. Extraction was made by autoclaving for fifteen minutes at fifteen pounds pressure, and the extracts obtained by filtration. The yeast juice was obtained as follows: three four-liter flasks, each containing three liters of WILLIAMS' solutions, were inoculated with a cake of Fleischman's yeast, and after a week the yeast was filtered from the solutions, autolyzed at 37° for twenty-four hours, and then dried by suction and washing with ether. Seventy gm. of yeast was then steamed for ten minutes with 250 cc. of distilled

water. The liquid was filtered and made up to a liter volume by the addition of distilled water.

All cultures were made in quadruplicate. The individual cultures of each series were strikingly uniform in growth, so that

TABLE I

Laelia-Cattleya HYBRID NO. 1;* SEEDS PLANTED AUGUST 31, 1920; MEASUREMENTS MADE JANUARY 27, 1921

CULTURE NO.	CULTURE SOLUTION	WIDTH OF EMBRYO IN MICRONS			PERCENTAGE OF GERMINATION	ORDER OF SUPERIORITY, MARCH 27
		Minimum	Maximum	Average		
B 53...	Full nutrient 50 cc.+50 cc. beet extract	242	582	407	0	5
B 17...	Full nutrient 50 cc.+50 cc. potato extract	339	630	459	0	5
B 60...	Full nutrient 50 cc.+50 cc. wheat extract	174	436	291	0	7
B 39...	Full nutrient+2% glucose	485	1261	970	20	4
B 28...	Full nutrient solution alone	194	242	213	0	8
B 27...	Full nutrient+2% glucose 90 cc.+10 cc. beet extract	485	1358	814	70	3
B 34...	Full nutrient +2% glucose 90 cc.+10 cc. wheat extract	582	1358	979	90	1
B 55...	Full nutrient+2% glucose 98 cc.+2 cc. yeast extract	582	1552	1076	90	1
B 66...	Full nutrient+2% fructose	776	1260	940	60	1
B 48...	Full nutrient+2% fructose 90 cc.+10 cc. beet extract	485	1358	902	80	2
B 22...	Full nutrient+2% fructose 90 cc.+10 cc. potato extract	485	1202	1008	90	1
B 31...	Full nutrient+2% fructose 90 cc.+10 cc. wheat extract	582	1260	902	70	2
B 5...	Full nutrient+2% fructose 98 cc.+2 cc. yeast extract	582	1358	1047	80	1
B 43...	Full nutrient+2% fructose 96 cc.+4 cc. yeast extract	582	1746	970	60	2
B 14...	Beet extract alone	242	872	388	0	5
B 69...	Potato extract alone	339	679	504	0	5
B 6...	Wheat extract alone	242	436	358	0	6
B 74...	Yeast extract alone	87	339	194	0	7

* Composition: *L. Perrinii* Lindl. ½; *C. labiata* Lindl. ½; *C. amethystoglossa* Reichb. ½; *C. intermedia* Grah. ½.

for the first measurements only one culture for each series was taken, and forty individual measurements were made for each culture. The measurements given in table I were made on January 27,1921. The order of superiority of the cultures on March 27 is recorded in the last column of the table. Similar data, not

included, were obtained in a like experiment with *Laelia-Cattleya* hybrid no. 2.

The degree of development represented by the numbers 1 to 8 is as follows: (1) dark green seedlings, most of these with two leaves and a few showing roots; (2) seedlings the same as no. 1, but light green; (3) seedlings light green, most of them with one leaf and a few showing two leaves; (4) seedlings light green, with only one leaf and that leaf short; (5) about 50 per cent of embryos showing leaf point; (6) embryos just showing a depression in meristem region; (7) advanced spherule stage; (8) smaller spherule stage.

The data in table I show that fructose is more favorable for growth of the embryos than glucose. This is apparent not only in the percentage showing leaves, but in the general appearance of the cultures. The embryos in the glucose cultures were whitish or yellowish in color. On the other hand, the fructose cultures were dark green. A more striking difference was noted on March 27, when in the glucose cultures the embryos were still yellowish and had shown no appreciable gain since January 27. The fructose cultures, on the other hand, had progressed and were still more markedly superior to the glucose cultures than on January 27. Fig. 1 shows the fructose culture and the nutrient solution culture minus sugar.

The addition of a plant extract to the glucose cultures has a marked effect on growth and chlorophyll development. In each case the percentage of germination is higher than with glucose alone, and the ranking of glucose-containing cultures on March 27 indicates that those with yeast or wheat extract rank with the best cultures, that with beet extract ranks in the third group, and the cultures with glucose alone fall in the fourth group. The addition of plant extract to the fructose-containing media is practically without any beneficial effect.

The loss or lack of development when glucose is supplied in the nutrient solution has been noted by MAZÉ and PERRIER (9) for corn, and by SERVETTAZ (10) in nutrition experiments with moss. In the case of orchid embryos the chlorophyll makes its appearance only when the leaf is developing, and then generally only in the leaf. Even then the leaves are only of a light green

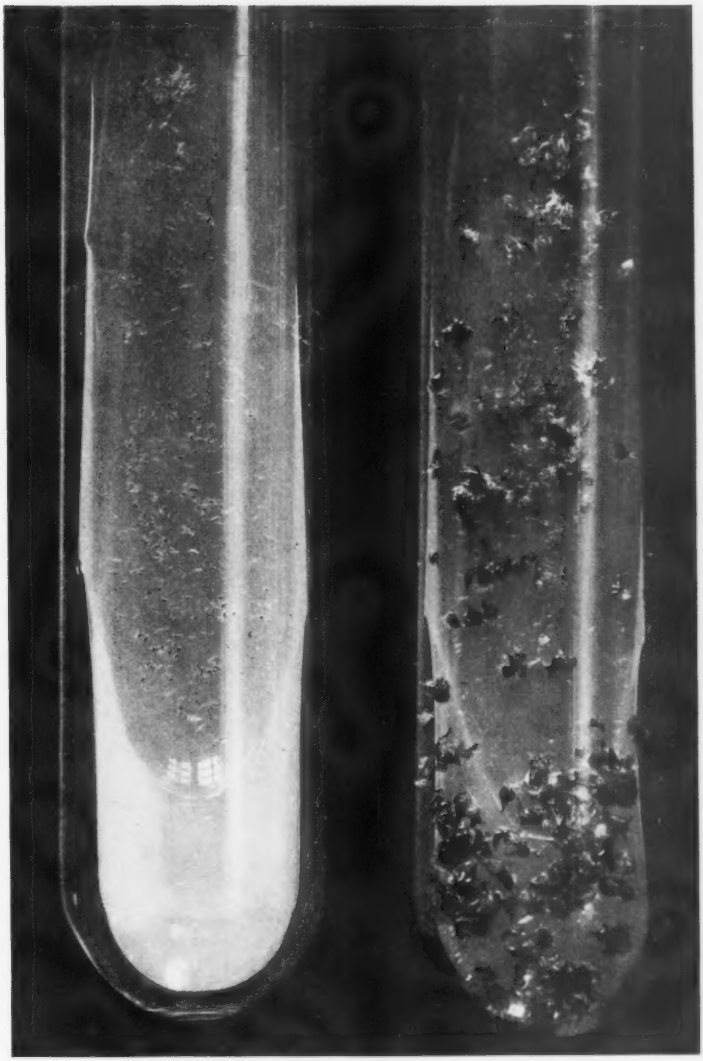

FIG. 1.—Solution B, embryos in small spherule stage; solution B+2 per cent fructose, seedling stage; ×2.

color. Is chlorosis due to a non-utilization of nitrogen or iron in the presence of glucose, or in the upbuilding of chlorophyll is glucose less favorable than fructose? Certain experiments now in progress may throw some light on this interesting point, and it is therefore desirable to await the results before speculating any further.

On January 27 the leaf point was not yet evident in any of the plant extract cultures. On March 10, however, in the wheat, beet, and potato extracts, embryos with leaf points were apparent, and a little later the same was noted for the yeast extract cultures. After several months more, seedlings with one and two leaves were to be noted in all these cultures.

Is germination on these extracts due to sugars or to other substances? Analyses made of the different extracts show that the potato, wheat, and yeast extracts had a sugar content of less than 0.025 per cent. The extracts diluted one-half with the nutrient solution, therefore, practically speaking had no sugar. The beet extract had a sugar content of 0.80 per cent, yet it did not permit any better germination than did the potato extract with merely a trace of sugar. As indicated previously, it should be borne in mind that the beet extract contains some substance injurious to the embryo. Furthermore, the stimulating effect of the plant extracts when added to the glucose solutions must be due to substances other than sugars. In the experiments on the influence of concentration of sugar, it will be noted that on the concentration of 0.05 per cent no germination has occurred even after four months.

Influence of concentration of sugar

In view of the fact that germination is possible when sugar is supplied in the culture media, it seemed desirable to determine the concentration most favorable for growth. Accordingly several series of experiments were made, a number of which are here reported.

In the first experiment seeds of *Laelia-Cattleya* hybrid no. 2 were used. These were planted November 12, 1920, and notes were made December 16, 1920, and January 11, February 15, and March 15, 1921. Each of the average figures given represents the average

of thirty separate measurements. The data are given in table II. The slow growth is well shown. There is in general a corresponding increase with increase in concentration, but the increase in concentration beyond 0.80 per cent is without any significant effect. On February 15 the embryos of all the cultures were examined for starch. It was found only in those cultures with 0.80 per cent glucose or higher. This fact is evidence that the absorption of glucose at a concentration of 0.80 per cent is in excess of the utilization, and consequently a higher concentration

TABLE II

INFLUENCE OF CONCENTRATION OF GLUCOSE, *Laelia-Cattleya* HYBRID NO. 2;* SEEDS SOWN NOVEMBER 12

CULTURE SOLUTION	AVERAGE WIDTH OF EMBRYOS IN MICRONS		
	December 16	January 11	March 15
Solution B	126	145	174
Solution B 0.05% glucose	184	232	247
Solution B 0.10% glucose	200	252	330
Solution B 0.20% glucose	242	281	475
Solution B 0.40% glucose	310	291	455
Solution B 0.80% glucose	291	339	533
Solution B 1.00% glucose	320	417	543
Solution B 2.00% glucose	320	436	523

* Composition: *C. Trianaei* Reichb. ½; *C. Loddigesii* Lindl. ¼; *L. purpurata* Lindl. ¼

should be without any increased beneficial effect. It should be borne in mind that glucose used with solution B is not particularly suited for the germination of orchid seedlings, since there is induced constantly in the embryos a distinct chlorosis. It is probable that higher concentrations of sucrose or fructose would permit of a more rapid germination.

The results of several other experiments on the influence of different concentrations of glucose on the germination of seeds of *Cattleya* are in agreement with these results, and need no repetition. In an experiment with seeds of *Epidendron*, germination was obtained with a concentration of 0.2 per cent glucose. In the cultures with less than 0.1 per cent glucose, not only was there a less development of the embryos, but a large percentage of the seeds never showed any initial swelling and development of chlorophyll.

The detailed data are given in table III. The figures given under average width represent the averages of forty individual measurements. Only seeds that had shown an initial increase in diameter and were green were included.

In this experiment another interesting observation was made. Just previous to the formation of the leaf point, the embryos were gorged with starch. With the formation of the leaf, however, there was a disappearance of the starch, it having been converted into

TABLE III

INFLUENCE OF CONCENTRATION OF GLUCOSE, *Epidendron tampense*×*E. inosmun;* SEEDS PLANTED DECEMBER 8, 1920; NOTES TAKEN MARCH 17, 1921

CULTURE SOLUTION	CULTURE NO.	WIDTH OF EMBRYOS IN MICRONS			PERCENTAGE WITH LEAVES	REMARKS
		Minimum	Maximum	Average		
Full nutrient.....	P 2	116	203	145	.....	95% no change
Full nutrient+0.05% glucose	P 72	116	291	178	.....	90% no change
Full nutrient+0.1 % glucose	P 78	189	407	281	.....	80 %no change
Full nutrient+0.2 % glucose	P 81	339	630	465	10	30% no change
Full nutrient+0.4 % glucose	P 86	291	582	397	10	40% no change
Full nutrient+0.8 % glucose	P 91	194	582	446	20	50% no change
Full nutrient+1.00% glucose	P 99	339	582	446	10	30% no change
Full nutrient+2.00% glucose	P 104	291	630	446	10	25% no change

sugar, as evidenced by the fact that some of the embryos still showed a slight presence of dextrins.

Influence of microorganisms

Throughout the various experiments made a few cultures always became contaminated. Generally, if the contamination was a *Penicillium*, the embryos became covered by the mycelium and death resulted. Those embryos not covered often showed a marked increase in growth over the embryos in the corresponding uncontaminated cultures. The increase in growth may have been due to one or a combination of the following: an increase in the carbon dioxide content of the tube; a change in the chemical character of the nutrient medium, brought about either by secretion of organic substances from the fungus or by products produced on decomposition of the fungus; or changes in the sugar effected by extracellular enzyme action.

In an experiment with seeds of *Epidendron* growing on solution B plus 0.80 per cent glucose, one of the cultures became contaminated with a species of *Actinomyces*. The result was that when the embryos in the contaminated culture were dark green, with one and two leaves, the embryos in corresponding uncontaminated cultures were still white or yellowish and only one or two of them showing the leaf point. In the contaminated culture the embryo

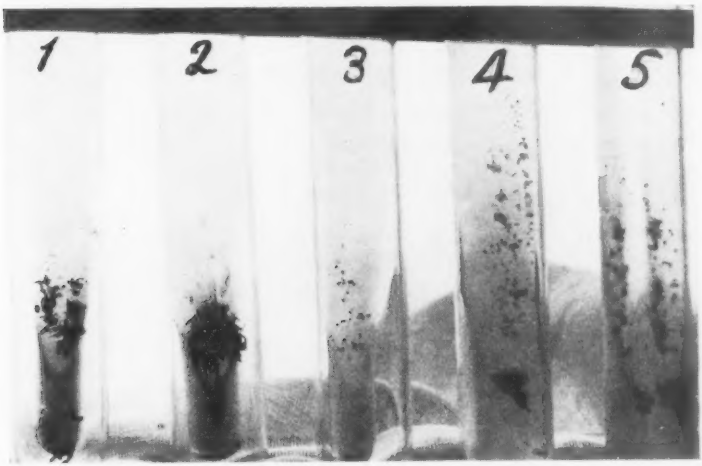

FIG. 2.—Culture no. 1, solution B+2 per cent glucose contaminated by *Actinomyces* sp. (corresponding check like no 3); culture no. 2, Pfeffer's +2 per cent sucrose inoculated with *Bacillus radicicola;* culture no. 3, same but not inoculated; culture no. 4, solution B+2 per cent glucose; culture no. 5, solution B+2 per cent glucose inoculated with *B. radicicola*; ×⅔.

had an average width of 975 μ, while in the uncontaminated cultures the average width of the embryos was 600 μ.

In view of these observations, and since BOTTOMLEY reported a beneficial effect on the growth of *Lemna* by the addition to the nutrient solution of an aqueous extract of *Azotobacter* or of *Bacillum radicicola*, an experiment was made to determine the influence of the latter organism on germination. Ten tubes were prepared with Pfeffer's solution plus 2 per cent sucrose; five were inoculated with *B. radicicola* from alfalfa, and five were left uninoculated. Seeds of *Epidendron* were sown on December 18, 1920. On March 5, 1921, 80 per cent of all the seeds in the inoculated tubes had

germinated and possessed one or two leaves. On the cultures not inoculated, the embryos lacked chlorophyll, and not one had produced even the leaf point. Most of the embryos exhibited the depression at the meristem region. Two months later some of the embryos, although still lacking chlorophyll, had formed the leaf point; while in the inoculated cultures the seedlings had two and three leaves and some had already produced roots (fig. 2).

The results appeared so unusual that the experiment was repeated with seeds of *Laelia-Catileya* hybrid no. 3, using solution B and also Ashby's solution. The composition of the nutrient

TABLE IV

INFLUENCE OF *Bacillus radicicola* ON *Laelia-Cattleya* HYBRID NO. 3;* SEEDS SOWN MARCH 14, 1921; NOTES TAKEN AUGUST 10, 1921

Nutrient medium	Average width in microns
Solution B	271
Solution B+0.1% glucose	407
Solution B+1.0% glucose	814
Solution B+inoculated	194
Solution B+0.1 glucose inoculated	378
Solution B+1.0% glucose inoculated	834
Ashby's solution	194
Ashby's solution+0.1% glucose	446
Ashby's solution+1.0% glucose	698
Ashby's solution+inoculated	164
Ashby's solution+0.1% glucose inoculated	397
Ashby's solution+1.0% glucose inoculated	970

* Composition: *C. superba* Schomb. ½; *C. Dormaniana* Reichb. ½; *C. Warscewiczii* Reichb. ½; *L. purpurata* Lindl. ½.

solution and the width of embryos are given in table IV. The favorable influence of *Bacillus radicicola* was noted only in the cultures having 1 per cent glucose. On solution B+1 per cent glucose the diameters of the embryos averaged the same for both the inoculated and the uninoculated seeds, but there was a striking difference in the color and the number with leaves. In the uninoculated, 20 per cent of the embryos were showing the leaf point, but the embryos were whitish in color. In the inoculated, 50 per cent of the embryos showed leaves, the leaf development was greater, and the embryos were green.

On Ashby's medium plus 1 per cent glucose, the beneficial effect of the organism was more apparent. There was a marked increase in the width of the embryos. In the inoculated cultures all the embryos had produced one or two leaves and the embryos were dark green. In the uninoculated cultures only 25 per cent of the embryos had produced a leaf point and the embryos were chlorotic (fig. 2). On both solution B and Ashby's+1 per cent glucose, the influence of *Bacillus radicicola* was so strikingly beneficial that it was observable immediately.

In the cultures with 0.10 per cent glucose or no glucose, the influence of *B. radicicola* seemed to be injurious, for in all the inoculated cultures the average width of the embryos was less than in the uninoculated cultures.

The cause of this favorable influence of *Bacillus radicicola* on the growth of orchid embryos remains yet to be determined. Some experiments were also made in which the cultures were inoculated with *Azotobacter* sp. In every case, however, there was a marked retardation in growth.

Transplanting experiments

On July 12, seeds of *Laelia-Cattleya* hybrid no. 2 were sown on solution B plus 2 per cent fructose plus 1.5 per cent agar. As culture vessels, Erlenmeyer flasks of 150 cc. capacity were used, and 30 cc. of the medium was employed. On October 14 the embryos were just on the verge of producing the leaf point. They were then transferred to six Erlenmeyer flasks (D 18 to D 23) containing 50 cc. of nutrient media, as follows: Pfeffer's solution+2 per cent glucose+0.1 cc. carrot decoction.

On March 1, 1920, the seedlings in cultures D 18 to D 23 had two and three leaves with a pronounced protocorm, and from the protocorm one and two roots had grown out, the roots varying from 1 to 3 cm. in length; whereas in the corresponding tube cultures, four seedlings in a hundred had produced roots, and these roots were only 2 mm. and 3 mm. in length. On March 11, 1921, seedlings were transferred from cultures D 22 and D 23 to liter Erlenmeyer flasks containing 300 cc. of solution B plus or minus sugar. Eight cultures were made, four with 2.0 per cent sucrose

and four without sugar. On August 20, 1921, notes were made on these cultures. In the sucrose cultures the seedlings had made a marked development. The largest seedlings had four and five leaves, some of the leaves being 2 cm. in length; while the roots of these seedlings, two or three in number, were 2–5 cm. in length (fig. 3). In the cultures lacking sugar the growth was less striking, the leaves being 2–4 mm. and the roots 2–10 mm. in length.

FIG. 3.—Seedlings one year old on solution B+2 per cent sucrose; $\times\frac{2}{3}$.

Seedlings were transplanted at the same time from D 23 to a compost of peat and sphagnum in ordinary flower pots. These seedlings, on August 20, 1921, showed leaves 5–7 mm. and roots 1–2 cm. in length. These seedlings were better than those planted on solution B in the liter flasks, but not so good as the seedlings on solution B plus 2 per cent sucrose in the liter flasks.

That better growth is possible in 150 cc. to 500 cc. Erlenmeyer flasks than in culture tubes was demonstrated repeatedly during these experiments. Erlenmeyer flasks of from 150 to 500 cc. capacity, containing the culture media left over after supplying the tubes with the requisite amount, were generally planted with the seeds that remained after the tubes were sown. In practically every instance germination took place sooner in the flasks than in the tubes. The probable explanation is that in the tube cultures the inward diffusion of carbon dioxide is impeded to a certain extent by the cotton plug, and, the volume of air in the tube being small, the carbon

dioxide is soon exhausted. In the larger flasks, however, the volume of air, and therefore the volume of CO_2, is much greater, from six to twenty-five times as great, and furthermore, the area through which the CO_2 can diffuse is greater by virtue of the larger mouths of the flasks. That the diffusion of CO_2 is impeded by a cotton stopper was shown in a previous paper (KNUDSON 7).

From the practical standpoint this would seem to be a method for the propagation of orchid seeds. The seeds may be germinated in the small culture tubes or in larger containers, and when roots are produced they may be transplanted either to pots in the open or transferred to sterile culture in larger flasks. My efforts to develop the seedlings on peat sphagnum mixture in flower pots in the open resulted in failure on several different occasions, due in one case to the temperature running up to 40° C., which permitted a pathogenic fungus to destroy utterly the seedlings in about twenty-five pots. In another case, during an absence from the city the seedlings were destroyed by insects. Previous to the misfortunes which the seedlings experienced they had been growing for periods of three and four months and were making satisfactory development. Other experiments are now in progress on this phase of the question. Some tubes were sent to Mr. T. L. MEAD, of Oviedo, Florida. Some of these were transplanted four and five months ago, and according to a recent communication from Mr. MEAD, the seedlings transferred are continuing growth, and but few seedlings were lost as a result of transplanting. The results of certain experiments now in progress indicate that more rapid growth will be obtained if the culture seedlings are transferred to sterile media containing sugar and grown for a year or two under these conditions. This method, moreover, has the advantage that the seedlings are not exposed to the depredations of insects or the ravages of parasitic fungi. Furthermore, contamination of the cultures by *Penicillium* or *Aspergillus* is without any injurious effect, provided that at the time of transplanting the seedlings have roots.

Discussion

What is the significance of these results in relation to the views advanced by BERNARD and BURGEFF, and quite generally believed

today, that for the germination of orchid seeds infection of the embryo by the appropriate fungus is essential? BERNARD believed that the action of the fungus was a physicochemical one, in that the fungus would cause an increase in a concentration of the cell sap, which increase in concentration would induce germination and the formation of a protocorm in somewhat the same way that the form of algae could change by increasing the concentration of the external solution. He points out that the fungus can invert sucrose and this may occur in the embryo.

The writer believes that the fungus may bring about germination in another way. As previously pointed out, in all his media BERNARD used a substance known as salep. This is a powder derived by grinding the dried tubers of certain species of orchids, and is rich in pentosans and starch, containing also about 5 per cent of organic nitrogenous substances. It probably contains some soluble organic and inorganic matter, judging from freezing-point determinations made by BERNARD. In view of the fact that organic matter is present, it is conceivable that the influence of the fungus might be to digest some of the starch, pentosans, and nitrogenous substances; which digestion products, together with secretions from the fungus or products produced on decomposition of the fungus, might be the cause of germination. In brief, it is conceivable that germination is induced not by any action of the fungus within the embryo, but by products produced externally on digestion or secreted by the fungus. Unfortunately I have not as yet succeeded in satisfactorily isolating the organism stated as necessary by BERNARD, nor has it been possible to purchase salep. Work is still in progress on this problem. There are, however, certain facts which support the idea that the action of the fungus is not necessarily internal. BERNARD does not give any analyses of the medium used, but he does give certain cryoscopic data. The medium generally used, made with salep, had a freezing-point depression of 0.01° C. Assuming that this depression ($\triangle$) is produced largely by hexose sugars, it would indicate at the outset of the experiment a concentration of hexose sugars equivalent to 0.1 per cent glucose. It is not possible, of course, to say that this depression is due entirely to hexose sugars; perhaps other sugars are present,

as well as other soluble organic and inorganic substances. The significant fact is that at the outset some soluble organic substances are present.

In addition to the soluble substances present, which apparently are not sufficient in quantity nor suitable as regards quality to permit of germination, there are to be considered the insoluble organic substances, pentosans, starch, and organic nitrogenous substances. Digestion of starch by the fungus would augment the concentration of sugar, and digestion of the organic nitrogenous substances might produce certain products which would make possible the germination of the seeds.

In my experiments it is true that the sugar used generally was of a relatively high concentration, but in the case of *Epidendron*, germination was obtained on 0.2 per cent glucose, which sugar is not particularly favorable for growth. That other substances besides sugar exert a pronounced influence is shown by the experiments on the beneficial effects of adding certain plant extracts to the glucose-containing solutions. The fact that other substances besides sugars may be important in the germination is shown by the experiments in which germination was obtained on decoctions of yeast, wheat grains, or of potato. All of these extracts contained less than 0.02 per cent total sugar. The experiments on the influence of *Bacillus radicicola* also lend weight to the idea that certain extraneous products may markedly influence germination.

BURGEFF, in certain of his experiments, used 2 per cent salep, but in other experiments he used starch, sucrose, or glucose. The explanations offered with respect to the function of the fungus in discussing BERNARD'S work may be used to account for the results obtained by BURGEFF. There may appear to be rather more difficulty in explaining the function of the fungus in the cultures containing either glucose or sucrose. It will be necessary to discuss these in more detail.

In one experiment, seeds of *Cattleya* were sown in a tube containing a nutrient solution plus 0.33 per cent sucrose. After three months the embryos were 0.4–0.5 mm. in width. Then, according to BURGEFF, they remained stationary. Cultures four months old, inoculated and maintained in the dark at 23° C., produced the first

leaf at the age of eight months. BURGEFF does not state that the uninoculated cultures were maintained under the same condition, but presumably they were. Granting that the uninoculated culture did not produce leaves, it is possible to explain the germination on the basis of the inversion of sucrose, which would yield approximately concentrations of both glucose and fructose molecularly equivalent to the original sucrose concentration. In addition there is to be considered the possible influence of products secreted by the fungus or produced on decomposition of the fungus.

The favorable influence of saprophytic fungi and bacteria demonstrated by my experiments is paralleled by certain cultures of BURGEFF. He transplanted four months' old seedlings to a mineral nutrient medium containing salep. Some of the cultures became contaminated with saprophytic organisms. The uncontaminated culture showed little growth, if any, after three months; while the culture contaminated by *Penicillium* made a marked growth, the leaves being 4 cm. in length. Another saprophytic fungus in another culture likewise caused a marked increase in growth, the leaves being about 9 mm. in length; and in a third culture contaminated by bacteria the seedlings were of similar character to those of the pure culture, but apparently darker green in color. All of these seedlings had produced vigorous roots. In the pure culture some of the seedlings had died. BURGEFF considers that the more favorable growth in the tube with fungus contamination was due to the development of an acid reaction in the medium. There is a possibility that increased carbon dioxide content and other products produced by the organism are partly responsible. It should be stated that the seedlings originally transferred to these tubes had previously been infected by the essential fungus.

Another experiment of BURGEFF lends weight, however, to the idea that the fungus is effective in inducing germination as a result of certain reactions brought about within the embryo. In this experiment the culture medium consisted of a weak nutrient solution plus $\frac{1}{2}\frac{1}{0}$ per cent starch. Seeds of a *Laelia-Cattleya* cross were planted, and the root fungus from each of seventeen different orchids was tested for its ability to induce germination. In the cultures uninoculated the embryos attained a width of 0.45 mm. in four months. In certain cultures only the suspensor became

infected. The diameter of the embryos in this case reached from 0.6 to 0.8 mm. in the same time. With infection still more advanced, but less than normal, a few seeds had leaves after seven months. Normally infected embryos produced leaves, and embryos that adhered to the wall of the tube likewise germinated. An infection more advanced than normal caused the same development as an infection slightly less than normal. When from one-half to two-thirds of the embryos became invaded, growth was less than in the embryos not infected, and in another case the seeds were killed outright.

As stated, certain facts from these experiments make it difficult to explain the action of the fungus as purely external. If so, why should the fungi behave so differently in inducing or retarding germination? Unfortunately BURGEFF gives no details so that one may judge whether or not these results could be duplicated on second trial. Another difficulty is an adequate explanation for the germination of seeds adhering to the inner surface of the culture tube. It is possible, of course, that decomposition products of the fungus growing on the surface of the tube may have been the cause. It is desirable to await experiments with the fungus before attempting to discuss these points further.

There are other phases of the problem presented by BERNARD, especially the loss by the fungus of its capacity to induce germination after prolonged culture in the laboratory. It is entirely possible that there has been no loss in the fungus, but that at the time of inoculating the culture the physiological state of the embryos was such as to resist or permit of infection. Those in which the infection was confined to the lower cell could still germinate despite the fungus. Those invaded to a greater extent would be killed. These and other experiments of BERNARD and BURGEFF suggest that one of the causes for the failure of germination is the parasitic character of the fungus. In other words, it is possible that the fungus, instead of being an aid in normal germination, is a factor in the death of the embryos and consequently in the failure of germination.

In conclusion, it may be stated that the evidence for the necessity of the fungus for germination has not yet been conclusively proved. The evidence is conclusive that under conditions of pure

culture employed by both BERNARD and BURGEFF germination of the seeds is dependent on the fungus. There is still considerable work to be done, however, before the validity of the fungus hypothesis can be proved or disproved.

Summary

1. A method is given for sterilizing seeds of certain orchids and for growing them under sterile conditions.

2. Germination of seeds of *Laelia*, *Cattleya*, and related forms is possible without the aid of any fungus when certain sugars are supplied.

3. Fructose appears more favorable than glucose.

4. In the presence of glucose, chlorosis of the embryo generally results.

5. Germination is possible on certain plant extracts containing merely traces of sugar.

6. Embryos in sugar-containing cultures accumulate a considerable reserve of starch.

7. The concentration of glucose is important in the growth of the embryo.

8. *Bacillus radicicola* from alfalfa and certain other microorganisms on certain media have a favorable influence on the development of chlorophyll and germination.

9. Seedlings have been transplanted from tubes to large flasks and growth has continued.

10. The results thus far obtained indicate that the method is of value in the propagation of orchids from seeds.

11. The idea is advanced that the necessity of fungus infection for germination has not yet been proved.

12. One cause of failure of germination may be the pathogenic character of some of the endophytic fungi.

Mr. T. L. MEAD, of Oviedo, Florida, who has worked for many years on the practical problems of germinating orchid seeds, has supplied all the seeds used in these experiments. He has likewise supplied certain information on the practical difficulties and on

various results obtained by him. For all of these favors and for constant interest I wish to express my thanks.

LABORATORY OF PLANT PHYSIOLOGY
CORNELL UNIVERSITY

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YELLOW-WHITE PINE FORMATION AT LITTLE MANISTEE, MICHIGAN

LEROY H. HARVEY

(WITH SIX FIGURES)

In a previous paper the writer[1] presented the inadequacy of current phytogeographical classification of the northern half of the Southern Peninsula of Michigan, and at that time advanced a scheme consonant with observational and experimental data. This region was shown to lie within that phytogeographical area whose climax (eschatophytic) forest type is of the maple-beech or mixed hardwood formation. It was shown that within this great transitional area there were at least two edaphic climax formation-complexes, the yellow-white pine and the black-white oak.

This paper is limited to a consideration of certain aspects of the "Big Pines" formation as it is exhibited at Little Manistee in Lake County, Michigan. The formation is now limited to two isolated stands one and one-half miles apart in the valley of the Little Manistee River which traverses each stand, the formation occupying its terraces and at a few points extending up on to the contiguous glacial highlands. These two stands are but the remnants of a once magnificent forest which occupied this general region. They are known locally as the Upper Pines and Lower Pines, and contain respectively about 160 and 60 acres. A general idea of these stands may be obtained from figs. 1–4.

During the preparation of this paper it was learned that the Upper Pines had suffered the inevitable, and that the saw and ax were rapidly resolving it into a historical fact. In light of this it has seemed wise to present rather fully all data available as a matter of record. Through the personal interest and activity of Judge HARRY D. JEWELL, of Grand Rapids, these magnificent stands of pines in 1914 were temporarily saved from complete deforestation, the logging operations actually being in progress at

[1] HARVEY, LEROY H., Some phytogeographical observations in Lake County, Michigan. Mich. Acad. Sci. Rept. 213–217. 1919.

the time of his intervention. Steps were taken toward the formation of a company to conserve this area, and the owner, E. GORDEN FILER, agreed to contribute a substantial part of the value of the standing timber. The State Legislature of 1915 sought to encourage the preservation of such tracts of timber, and passed a bill with the amendment to this effect, as proposed by Judge

FIG. 1.—Upper Pines from west at edge of glacial upland: shrubby-herbaceous association of reforestation occupies foreground; this will terminate in black-white oak complex.

JEWELL. Unfortunately the Governor, through the mistaken advice of one of the State Tax Commissioners who did not understand the merits of the measure, misconstrued its purpose, and vetoed the bill. It was hoped that this tract would eventually be made a part of the Public Domain. Action was delayed, however, and Michigan's archaic and prodigal method of forest taxation left no alternative. The Upper Pines are gone; a crime to be laid at the door of our state officials. Nevertheless, the efforts of these public spirited men cannot be commended too highly, and should

serve as a stimulus for other efforts all over the state looking toward the preservation in Public Domain of original stands of forest types and plant associations. This is not wholly a matter of nature sentiment, but one of scientific necessity.

FIG. 2.—Upper Pines, view within formation which here occupies a high terrace: dominant form of herbaceous layer is *Pteris aquilina;* atmometer station 7.

To Judge JEWELL I wish to express my deep appreciation of his hospitality during the prosecution of my field work. I also wish to express my thanks to my colleague, Dr. WILLIAM MCCRACKEN, who assisted me throughout in taking data.

Climatic factors

The climatic conditions of the region in all probability are fairly revealed in the data taken at Luther (table I), less than ten miles to the southeast, at a United States Volunteer Station. It will be noted that the maximum range in temperature for the four years is 136°, from 100° in August 1918 to −36° in February 1918. The average yearly rainfall for the four years is 33.65 inches, ranging from 28.29 inches in 1917 to 39.08 in 1920. The last killing frost in spring was on June 23, 1920, and the first killing

TABLE I

CLIMATIC FACTORS 1917–1920*

Factors	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Mean maximum temperature	24.2	26.4	42.3	56.1	66.8	77.5	80.7	78.6	70.1	54.6	43.7	30.8
Mean minimum temperature	8.45	7.7	21.0	30.7	40.7	52.0	55.1	53.5	46.2	39.6	28.9	16.8
Maximum temperature.....	45	45	68	72	92	92	98	100	89	80	65	65
Minimum temperature.....	−27	−36	−18	11	22	27	33	34	27	18	6	−19
Mean precipitation	1.37	1.07	2.49	2.93	3.35	3.38	3.68	3.49	3.06	4.00	2.44	2.38
Minimum precipitation.....	1.07	0.65	1.50	2.45	2.03	2.00	1.36	2.23	2.32	3.09	1.17	1.28

* The temperature records are in Fahrenheit and the precipitation in inches.

frost in autumn was on September 11, 1917. The shortest growing period was 87 days in 1917, and the longest 106 days in 1919–1920, with an average of 98 days. While means and averages convey certain pertinent facts, it is the extremes which indicate the critical points of climatic influence on vegetation.

These climatic conditions are adequate to support the maple-beech or mixed hardwood formation which are found in typical development within the region. It is apparent, therefore, that the yellow-white pine complex is the expression of a combination of edaphic factors which exclude the climax type. The main critical factor, aside from the question of post-glacial invasion and pre-occupation, is undoubtedly the wilting coefficient of the soil.

Ecological factors

The ecological factors investigated included soil moisture, evaporation, and light intensity. Records were made over a period of twenty-eight days, August 3–31, 1918. As August–September practically represents the minimum of favorable ecological conditions for the growing period, the factorial values of either month

FIG. 3.—Lower Pines, northern end of stand: white oak in left foreground; reforestation from white oak grubs and invasion of black oak.

FIG. 4.—Lower Pines: fires excluded for a number of years, and there is a well developed shrubby layer in which species of *Cornus*, *Viburnum*, *Amelanchier*, *Crataegus*, and *Prunus*, as well as saplings of red and white oak are common; seedlings and saplings of white and yellow common in open places.

will adequately serve to indicate the critical ecological conditions. It was expected that some light would be thrown by such data upon the questions of the relation of evaporation to succession and upon the classical conception of the successional development of the formations of a region. In my paper already referred to, the data seemed to show that evaporation had no causal relation to succession. It was argued that the measurements of the ecological factors of any association are more largely a result of that stage of the succession rather than the cause of it. Specifically, the evaporation is less in the Big Pines formation than in the open herbaceous association, because of the greater protection in the former from factors enhancing water loss; the Big Pines formation is the cause of lowered evaporation and not the result of it. The evaporation conditions of any pioneer association obviously represent the conditions which must be tolerated by the invaders from any higher ecological type of association. These invaders help to reduce the evaporation, and finally, as the next step in the succession is established, it comes to possess its own evaporation ratio determined by its own canopy, shrubby, and herbaceous layers.

SOIL WATER.—Soil samples were taken in two series at depths of 7.5 cm. and 25 cm. by means of a knife-blade trowel, and placed at once in friction top cans of 350 cc. capacity. The samples were collected on August 4, 11, 18, and 25. Analyses of the samples were completed within the following month. The moisture-holding capacity (MHC) was computed upon the basis of dry weight as well as upon the basis of equal volumes, according to HILGARD'S[2] method. Each determination represents the average of the four samples for each station and series. The total field capacity (TFC) was computed directly upon the basis of dry weight. The wilting coefficient (WC) was calculated by the formula of BRIGGS and SHANTZ.[3] $WC = \frac{\text{moisture holding capacity} - 21}{2.90(\pm 0.021)}$, in which the MHC is determined by volume per cent. It is recognized that the WC data thus computed are only an approximation, as such data preferably should be based either upon experimental

[2] HILGARD, E. W., Soils. New York. 1906.

[3] BRIGGS, L. J., and SHANTZ, H. L., The wilting coefficient for different plants and its indirect determination. U.S. Dept. Agric., Bur. Plant Ind. Bull. 230; BOT. GAZ. 51:210–219. 1911; 53:20–37, 229–235. 1912.

determination or computed from the moisture equivalent mechanically determined. These data will be found in table II. For purpose of comparison similar data from the jack pine, black-oak, and the Kalamazoo maple-beech associations are added.

A study of these data brings out certain facts which are quite in agreement with field observations. The range in TFC in the Big Pines at 7.5 cm. from 7.1 to 14.9 per cent, with a corresponding range in WHC (column 3) from 48.5 to 52.5 per cent, is a direct expression of the effect of fires on the humus layer. At 25 cm. this effect is less apparent in the WHC, but the absence of protection from a good humus layer is shown in the TFC at the same depth.

TABLE II

SOIL WATER DETERMINATIONS IN PERCENTAGES

Station and association	TFC by DW		WHC by DW		WHC by volume		WC by formula	
	7.5 cm.	25 cm.	7.5 cm.	25 cm.	7.5 cm.	25 cm.	7.5 cm.	25 cm.
6. Upper Pines	14.9	6.6	44.4	28.7	53.5	41.8	11.2	7.1
7. Upper Pines	9.9	5.3	43.4	38.2	52.3	43.4	10.8	7.5
9. Upper Pines	7.1	4.3	36.3	30.5	48.5	43.1	9.5	7.5
5. Jack pine	14.7	6.1	50.9	34.9	60.4	42.7	13.6	7.5
3. Black-white oak	6.4	4.7	33.1	30.1	45.2	42.7	8.4	7.5
Maple-beech (Kalamazoo)	.....	.....	62.1	33.4	62.7	45.7	14.5	8.5

The soil is often so baked that it is grayish and "dead" to a depth of 4 or 5 cm. These oft repeated fires must have had marked influence upon growth increment of the facies, as well as largely determining the presence or absence and nature of the shrubby, herbaceous, and ground layers. The WC (column 4) exhibits in another way the same regressive influence due to humus destruction, producing a soil condition which must act selectively upon the various invading propagules. The uniform WHC and WC at 25 cm. in the Big Pines, as well as in the jack pines and oak associations, are very significant and form the evidence for the conclusion advanced in the former paper that the soil conditions reveal no differences of causal magnitude, and are thus clearly inadequate to explain present forest distribution, or to give any indication of a causal successional factor.

Fig. 5 is an attempt to exhibit to scale the soil-water relations in the Big Pines formation as they existed in August 1918. That the data may be comparable they are all computed on the basis of dry weight. The critical factor is the growth-water, whose theoretical possible maximum would be the difference between the WC and the WHC, or 29 per cent. This of course would scarcely ever be realized under field conditions. Actually the growth-water is the difference between the TFC and the WC, which in August 1918 was only 1.5 per cent, a quantity dangerously near the zero point of available water.

Evaporation.—The evaporation data were obtained in the usual way, Livingston standardized 8 cm. atmometers being used. During the month 284 mm. of rain fell on seven days, 250 mm. of

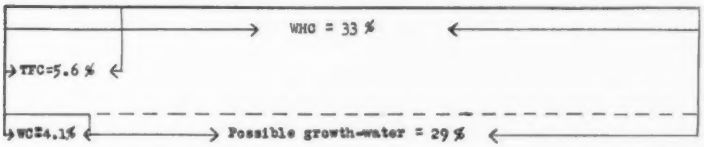

Fig. 5.—Soil-water relations (shown to scale): data on basis of dry weight.

this falling in twenty minutes. Rain correcting atmometers were not available, but it is thought that the use of these would not have given essentially different results. For five of the stations the records were taken daily; for the others every seven days. The 250 cc. reservoir bottles were all set slightly in the ground and stabilized with 12 inch length of $\frac{3}{16}$ inch mile steel to which they were bound by two bands of electric tape. Readings were made on an index mark on the neck of the bottle, and loss of water was supplied from a 50 cc. burette. Distilled water was used throughout. Stations were selected to represent various conditions of cover and canopy. The results are tabulated in table III.

The records in table III give evaporation rates only for the low herbaceous layer, and the average daily range from 8.3 to 18.6 cc. (124 per cent) is solely an expression of the degree of exposure, varying directly with the amount of insolation afforded by the herbaceous and shrubby layer. In connection with the

record of station 1*B* (18.6 cc.) and of station 1 (19.5 cc.), a difference of only 0.9 cc. (4.3 per cent) would seem to indicate that the canopy exercises only a slight effect in reducing evaporation from the herbaceous layer. "On the basis of these results it would appear entirely unwarranted to assign evaporation as a causal factor in succession."

LIGHT.—The light determinations were made with a Waynne's Infallible Meter, as this was the only device available. All readings seek to represent the maximum shade conditions due to the canopy; all part shadows due to the trunks were avoided. The

TABLE III

AVERAGE DAILY EVAPORATION IN CC., AUGUST 4–31, 1918

Station	Association	Number of atmometers	Evaporation (cc.)	Average for Big Pines (cc.)
1*B*.....	Lower Pines, B.	1	18.6	.....
1*C*.....	Lower Pines, C.	1	8.3	.....
2.....	Lower Pines Terrace	2	10.4	.....
6.....	Upper Pines, W. C.	2	12.5	.....
7.....	Upper Pines, S.	2	8.7	.....
8.....	Upper Pines, E. T.	2	14.7	.....
9.....	Upper Pines, E. C.	2	16.8	12.79
5.....	Jack pine	2	12.7	.....
3.....	Black-white oak	2	19.0	.....
1.....	Open-standard	1	19.5	.....
11.....	Garden, Kalamazoo	1	31.6	.....

meter was placed upon the ground and the stopwatch held behind the back; both were released simultaneously. When the solio paper merged imperceptibly into the darker standard, the watch was stopped and the reading recorded. The results represent the averages of eight or ten readings (table IV). All light data are referred to full sunlight, which for convenience of comparison are reduced to the standard one, and all habitat records are correspondingly reduced to the same standard and expressed in the form of a ratio. Thus in station 1 the canopy reduces the sunlight to one-fifteenth its full intensity.

The range in the light ratios is largely a question of density of stand and so density of canopy. It is interesting to note that the yellow pine canopy with the same density of individuals produces a light screen of only about one-half the value of that of the white

pine canopy. It would be of interest to know whether these shade differences are correlated with floristic or vegetational differences. This problem unfortunately escaped attention in the field.

Growth forms

Since the appearance of RAUNKIAER'S work in 1916, ecologists have viewed the influence of temperature factors from a radically new viewpoint. He points out that climatic conditions are reflected essentially in the biological nature of the vegetation, that is, in the nature and extent of the protection possessed by the perennating growth points during the winter or critical season. Upon the basis of this generalization he classifies vegetation into a series of

TABLE IV

LIGHT DETERMINATIONS, BIG PINES FORMATION

Station	Association	Ratio
1*B*	Lower Pines	1:15
2*A*	Lower Pines	1:12
7	Upper Pines	1:63
7	White pine canopy	1:57
7	Yellow pine canopy	1:30

life (or growth) forms. The following characterization of life forms and the abbreviations used are added for convenience of reference.[4]

Phanerophytes.—Woody plants of all types, both evergreen and deciduous, and exhibiting the least amount of protection from the cold, as showing the greatest amount of exposure. The group may be divided into *Megaphanerophytes* (MG), trees over 30 m.; *Mesophanerophytes* (MS), trees 8–30 m.; *Microphanerophytes* (MC), shrubs or trees 2–8 m.; *Nanophanerophytes* (N), shrubs under 2 m.

Chamaephytes (CH).—Perennial by virtue of the fact that the buds are just above the ground, or on the surface, and are thus often protected by the snow blanket.

Hemicryptophytes (H).—With dormant buds in the upper crust of the soil, the top of the plant dying down in winter.

[4] TAYLOR, N., The growth forms of the flora of New York and vicinity. Amer. Jour. Bot. 2:23–31. 1915.

Geophytes (G).—Perennial by bulbs, rhizomes, tubers, or by root buds.

Helophytes and *Hydrophytes* (HH).—The former has buds at the bottom of the water. Hydrophytes have perennating rhizomes or winter buds and are truly aquatic.

Therophytes (T).—Annuals.

On the basis of 400 species carefully selected from 1000 representative species, RAUNKIAER prepared what he calls a "normal spectrum," which shows the percentage distribution of these 400 species into the several life forms. This is now accepted as a basis of comparison.

The areas contiguous to the Big Pines have long been cleared, and are now covered with either grassy or shrubby plains or second growth oak associations. Considerable invasion has naturally occurred from these surrounding regions. Also repeated ground fires have run their course through both stands, although a part of the Lower Pines has been spared for several years. The north exposure of the immediate river banks furnishes many interesting isolated colonies of glacial relicts, but these, as well as the evident invasions, have been eliminated in the summary of species. An attempt has been made to include only those species which are evidently the natural components of the formation. Obviously this introduces the unchecked personal factor, and doubtless some errors of judgment have been made, but it is hoped not enough to essentially modify the results. As the study was made in August, doubtless some spring forms may also have been missed. On the basis of four separate lists, however, 100 species have been included (table V). In table VI the ecological spectrum of these 100 species is recorded. RAUNKIAER'S normal spectrum and TAYLOR'S (see footnote 4) spectrum of the northern elements of the flora within 100 miles of New York City are added for comparison. It should be noted further that only twenty-seven species have not been recorded outside of the Big Pines formation (table V). It is highly probable that careful search would further reduce this number.

A survey of the ecological spectrum of the Big Pines indicates that the mesophanerophytes determine the facies of the formation.

TABLE V

SPECIES OF THE BIG PINES FORMATION

Species	MS	MC	N	CH	H	G	T	Peculiar to form
Pteris aquilina*						×		
Pinus resinosa	×							
Pinus Strobus	×							
Agropyron tenerum						×		
Bromus ciliatus						×		
Bromus Kalmii						×		
Calamagrostis canadensis						×		×
Danthonia spicata						×		
Deschampsia flexuosa						×		
Koeleria cristata						×		
Melica striata						×		×
Muhlenbergia racemosa						×		
Oryzopsis pungens						×		
Panicum latifolium						×		
Poa triflora						×		×
Maianthemum canadense						×		
Polygonatum biflorum						×		
Smilacina racemosa						×		
Smilax hispida			×					
Cypripedium acaule						×		
Cypripedium hirsutum						×		×
Epipactis pubescens						×		×
Salix humilis			×					
Myrica asplenifolia			×					
Quercus alba	×							
Quercus rubra	×							
Actaea alba					×			×
Aquilegia canadensis					×			
Hepatica triloba				×				×
Arabis brachycarpa							×	
Ribes Cynosbati			×					×
Hamamelis virginiana		×						
Amelanchier canadensis		×						
Amelanchier oblongifolia		×						
Amelanchier spicata		×						×
Crataegus no. 1		×						
Crataegus no. 2		×						
Crataegus no. 3		×						
Fragaria virginiana				×				
Potentilla canadensis					×			
Prunus americana		×						
Prunus serotina	×							
Prunus virginiana		×						
Rosa humilis			×					
Rubus allegheniensis			×					
Rubus Idaeus			×					
Rubus triflorus			×					
Lespedeza procumbens							×	×
Xanthoxylum americanum			×					×
Polygala paucifolia						×		×
Acer rubrum	×							

* The nomenclature and sequence of the seventh edition of GRAY's *Manual* have been followed. Specimens are preserved in the author's private herbarium.

TABLE V—*Continued*

Species	MS	MC	N	CH	H	G	T	Peculiar to form
Acer saccharum	×							×
Ceanothus americanus			×					
Viola arenaria						×		
Viola pubescens						×		×
Viola scabriuscula						×		
Aralia nudicaulis						×		×
Sanicula gregaria					×			×
Cornus circinata		×						×
Cornus paniculata		×						
Arctostaphylos Uva-ursi				×				
Chimaphila umbellata				×				
Epigaea repens				×				
Gaultheria procumbens					×			
Gaylussacia baccata			×					
Pyrola americana						×		
Pyrola secunda						×		
Vaccinium pennsylvanicum			×					
Trientalis americana					×			
Asclepias purpurascens						×		×
Monarda mollis					×			
Prunella vulgaris					×			
Satureja vulgaris				×				
Melampyrum lineare					×			
Pedicularis canadensis				×				
Galium pilosum					×			
Mitchella repens				×				×
Diervilla Lonicera			×					
Lonicera canadensis			×					×
Lonicera glaucescens			×					×
Sambucus racemosa			×					×
Triosteum aurantiacum					×			×
Viburnum acerifolium			×					×
Viburnum pubescens			×					×
Campanula rotundifolia				×				
Antennaria neodioica				×				
Antennaria Parlinii				×				
Antennaria plantaginifolia				×				
Aster laevis					×			
Aster Lindleyanus					×			
Aster macrophyllus					×			×
Helianthus mollis					×			
Hieracium canadense					×			
Hieracium longipilum					×			
Hieracium venosum					×			
Prenanthes trifoliolata					×			
Solidago caesia					×			
Solidago hispida					×			
Solidago rugosa					×			×
Solidago serotina					×			
Total: 100 species	7	11	18	11	24	27	2	27

It has been noted that the influence of oft repeated fires regulates the "presence or absence and nature of the shrubby and herbaceous layers." The large percentage of hemicryptophytes and geophytes in a forest formation are thought to be in large part an expression of this influence.

Valences

The biological spectrum furnishes one of the essentials in the ecological characterization of a formation. The method of valences also offers an objective means of presenting an equally important picture, that of the numerical abundance of the various floristic elements of the formation. It is the frequency percentage of the

TABLE VI

PERCENTAGES OF GROWTH FORMS IN BIOLOGICAL SPECTRA

Type of growth form	MG	MS	MC	N	CH	H	G	HH	T
Big Pines.....	0	7	11	18	11	24	27	0	2
Normal spectrum.	7		17	20	9	27	3	1	13
New York City...	0	1.31	3.94	8.55	8.55	26.31	24.34	23	3.94

various elements which determines the aspect of the formation or its physiognomic character.

Various methods have been employed since the introduction of the quadrat method in such statistical investigation. The method used in this study in an adaptation of that used by RAUNKIAER.[5] As only the facies was considered, it was possible to devise a rapid method of record. A rope 5 m. long held in the hand of an assistant formed the radius of a circle whose species were recorded as the investigator moved the radius through 360°. The center of the next area was obtained by swinging the radius through 180°, and with this as a fixed point the assistant then took his stand 5 m. farther on in the same axis. Thus areas are tangent to each other. In this way a transect of 200 m. was run. The valence records are thus based upon twenty such areas (table VII).

[5] For accounts of RAUNKIAER's work see: SMITH, W. G., RAUNKIAER's life forms, and statistical methods. Jour. Ecol. 1:16–26. 1913; and FULLER, G. D., and BAKKE, A. L., RAUNKIAER's "life forms," "leaf classes," and statistical methods. Plant World 21:25–37. 1918.

The valence table shows that the yellow pine is slightly the dominant species in the Upper Pines. In the Lower Pines the reverse is true. It will be noted that this is due to the grouping of individuals of a species in certain parts of the stand, as is shown by transects IV and V. Similar grouping of the white pine is shown in transects I and II, while transect III shows an almost equal abundance of the two dominant species. These facts of abundance are not correlated with any soil differences as far as could be determined. It probably represents the influence of

TABLE VII

VALENCE OF FACIES IN FREQUENCY PERCENTAGE, UPPER PINES

SPECIES	TRANSECT NO.					TOTAL	RATIO
	I	II	III	IV	V		
Yellow pine	25	27	50	72	54	228	100
White pine	45	49	53	26	37	210	92
White oak	6	15	4	5	4	34	15
Red oak	0	1	0	0	0	1	0.4
Red maple	0	0	1	0	1	2	0.8
Total	76	92	108	103	96	465	.....

persistent seed trees of the original stand which predominantly determined the nature of the succession within their respective areas.

The red and white oaks are rather constant members of the formation, the white oak always predominating. While they do not influence the physiognomy of the facies, yet in case of cutting or burning, the white oak is the persistent species and largely determines the nature of the reforestation, giving rise to the oak or mixed oak formation in which the red oak fails to reappear, being replaced by the black oak and in some cases by the additional invasion of the jack pine, neither of which, so far as observed, is ever found in the Big Pines formation. The red maple represents sporadic invasion from the not far distant margin or floodplain of the Little Manistee River. It was not recorded from the upland portions of the formation.

Age distribution of facies

The ecological spectrum (table VI) and valences (table VII) convey a very definite concept of the Big Pines formation. Nevertheless the picture is incomplete. It is to supply this deficiency in the visualization of the formation that the following method of analysis has been devised. It is essential that the age of the facies and the relative abundance of the individuals of each age be known, for therein is recorded not only the present status, but much of the

TABLE VIII

AGE DISTRIBUTION OF FACIES, UPPER PINES, STATIONS 6 AND 7

Circumference classes in inches	10–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70
Pinus resinosa	4	6	7	5	8	6	11	6	6	....	....	.....
Pinus Strobus	2	7	8	8	3	6	6	6	7	2	....	1
Quercus alba	....	....	3	3	....	1	....	2	1	....	....	1
Quercus rubra	....	....	....	....	....	....	....	2	....	....	1	.....
Acer rubrum	....	....	....	....	1	....	....	....	....	....	....	.....

Circumference classes in inches	71–75	76–80	81–85	86–90	91–95	96–100	101–105	106–110	111–115	116–120	Total	Ratio
Pinus resinosa	1	1	2	....	....	....	....	....	....	....	63	100
Pinus Strobus	....	....	....	....	1	1	....	....	....	1	59	92.6
Quercus alba	....	....	....	....	....	....	....	....	....	....	11	17.2
Quercus rubra	....	....	....	....	....	....	....	....	....	....	3	4.7
Acer rubrum	....	....	....	....	....	....	....	....	....	....	1	1.5

past history, as well as the future ecological tendencies of the formation.

The data were obtained by running transects through several portions of the formation, measuring the circumference (BH) of the trees as met. Numerous cores were taken with an increment borer in order to establish an increment factor which could be used to convert the circumferences into age equivalents. This, however, gave no ratio entirely satisfactory for all sizes, probably due to too few samplings. It is believed, however, that the circumference data reveal the relative age distribution, although actual age determinations are greatly to be preferred. Several transects gave essentially identical results. The record of one of these transects, run

in a portion of the formation where the shrubby layer was entirely lacking, is shown in table VIII.

Fig. 6 reveals the facts in an even more striking way. Before considering this graph of the age distribution, it should be stated that the five inch circumference classes are arbitrarily chosen. Each class represents approximately fifteen years. If actual age determinations were at hand, it would be desirable to use a one year class, thus more exactly recording the facts. It is thus

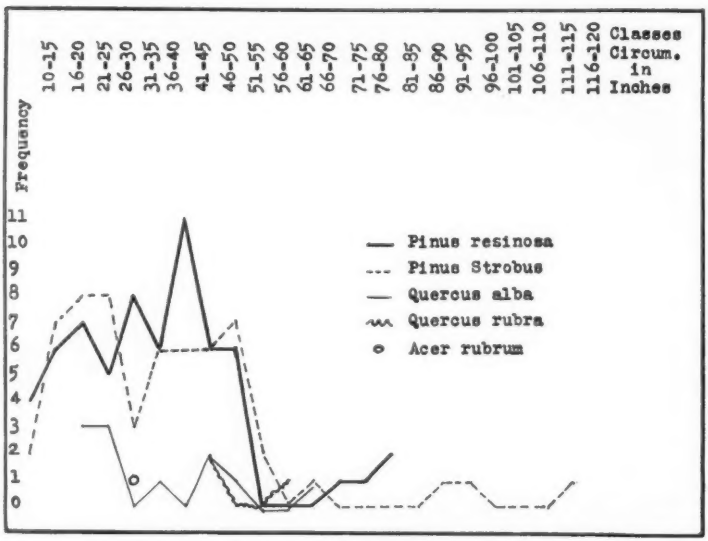

FIG. 6.—Age distribution of facies, Upper Pines, stations 6 and 7.

realized that the conclusions drawn from the graph are perhaps more valuable as a suggestion of a valid method of analysis than as a scientific representation of the actual conditions. It will be noted that the ratios of the three principal species of the facies (table VIII) are in almost perfect agreement with the ratios of frequency percentages as shown in table VII. It would appear that the transect analyzed is fairly representative of the formation.

Fig. 6 shows that the great mass of the individuals of the yellow pine lie between circumferences of 10 to 55 inches, which represents an approximate age range of 30–175 years. There are a few isolated

individuals whose circumferences (75–85 inches) indicate an age range of 240–275 years. The graph shows three fairly well marked modal points which are thought to represent periods of more abundant seeding or ecesis. With actual age data these periods could be accurately indicated as to years.

The white pine shows essentially the same range in age, 30–190 years, with two modal points less clearly established. Taking both stands into consideration, there are a few isolated ancient trees of white pine not included in the transect, whose circumferences measure 100, 115, 134, and 146 inches, and whose ages range from 350 to 450 years.

The total absence of seedling and sapling pines in this transect is probably due to the influence of shade and fires, for in certain open stations protected from fires for a period of years germination of both species appears to be common and reforestation is in process.

Consideration has already been given to the white oak, red oak, and red maple in a previous section.

On the basis of the preceding data one may venture to reconstruct somewhat the history of the formation. The original stand of the Big Pines would appear to extend back some 400 or 450 or more years, and doubtless the formation had then been self-perpetuating for centuries. The break in age between these persisting patriarchs and the present stand, whose mass falls under 200 years of age, is very striking, although it is bridged somewhat by a broken series of isolated trees. What is the explanation of this break in history? The date of the original logging of this region would not appear to account for this hiatus. It would rather seem that there had been an almost entire destruction of this formation some 200 years ago, possibly through fire of lightning origin, which then became reestablished through a few isolated individuals of various ages escaping the devastation. The well distributed range of ages down to 30 years would apparently indicate that the formation has been and is under natural conditions permanently self-perpetuating. It represents an ecological climax (eschatophytic) formation.

WESTERN STATE NORMAL COLLEGE
KALAMAZOO, MICH.

FORMULAS FOR CALCULATING NUMBER OF FRUITS REQUIRED FOR ADEQUATE SAMPLE FOR ANALYSIS[1]

F. E. Denny

When taking samples of variable fruits, as oranges for example, it is important to obtain an approximation of the number of fruits that should be included in the sample, in order that the results of the analyses shall be sufficiently accurate for the purpose of the investigation. It is the object of this paper to give formulas which may be used in such cases; to illustrate their use by numerical examples; to indicate the reliability that may be placed upon them; and to show the results that were obtained in applying them to the analysis of citrus fruits.

The first step consisted in obtaining a measure of the variability of the fruit in question. In the case of citrus, this was accomplished by analyzing individual fruits, since one fruit was found to yield enough material for the analytical work performed. If smaller fruits, such as plums, were used, it would be necessary to increase the sample to half a dozen, or a dozen, or some other number that would make a convenient sample with which to work, but the results of the analysis of each of the chosen units should be tabulated separately. From these data the probable error of a single sample was found, and this value formed the starting point for the calculations made in formulas described in later paragraphs.

Variability in composition of individual oranges in single sample

Fifty-one oranges were taken at random from a single tree. These fruits were all of good marketable quality, and were apparently free from diseases, insect injuries, and bruises. They were uniform in color, but of course variable in size. The fruits were analyzed individually and the results for each fruit tabulated

[1] Published by permission of the Secretary of Agriculture.

separately, as given in table I. At the bottom of the table will be found the values for the probable error of the mean and the probable error of a single observation. These were calculated from the following formulas: P.E. mean $= \pm 0.6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}$; P.E. sing. $= \pm 0.6745\sqrt{\frac{\Sigma d^2}{(n-1)}}$; where "n" is the number of variates (in this

TABLE I

COMPOSITION OF FIFTY-ONE ORANGES, WASHINGTON NAVEL VARIETY

Orange no.	Degrees brix	Percentage of sugar	Percentage of acid	Sol. sol. / acid ratio	Orange no.	Degrees brix	Percentage of sugar	Percentage of acid	Sol. sol. / acid ratio
1	12.80	9.63	0.98	13.05	30	13.70	10.91	1.07	12.80
2	13.10	10.30	0.98	13.35	31	14.00	10.93	0.96	14.60
3	12.50	9.46	1.08	11.60	32	13.70	10.68	1.14	12.00
4	13.70	10.44	1.14	12.00	33	15.30	11.92	1.15	13.30
5	14.40	11.17	1.06	13.60	34	13.85	10.83	1.06	13.05
6	15.00	11.14	1.06	14.15	35	13.20	10.31	1.02	12.95
7	13.90	10.85	0.84	16.55	36	14.70	11.51	0.86	17.10
8	13.40	10.43	0.98	13.70	37	14.75	10.96	1.04	14.20
9	13.70	10.94	0.93	14.75	38	15.30	11.57	1.29	11.85
10	13.70	10.65	0.84	16.30	39	15.30	11.46	1.23	12.45
11	13.55	10.71	0.90	15.05	40	13.75	10.88	0.91	15.10
12	13.35	10.14	1.15	11.60	41	13.40	10.18	1.29	10.40
13	13.20	10.35	0.94	14.05	42	13.35	10.33	1.19	11.20
14	13.95	10.85	0.98	14.25	43	13.45	10.09	1.24	10.85
15	14.30	10.83	0.96	14.90	44	14.45	11.00	0.94	15.35
16	15.05	11.59	0.95	15.85	45	12.80	10.05	1.15	11.15
17	14.90	11.80	1.02	14.60	46	14.00	10.76	1.27	11.00
18	13.20	10.30	1.09	12.10	47	14.70	11.26	0.86	17.10
19	15.25	12.05	1.00	15.25	48	14.90	11.44	0.98	15.20
20	13.40	10.53	1.02	13.15	49	12.60	11.35	1.07	11.80
21	14.85	11.18	1.11	13.40	50	14.80	10.88	1.31	11.30
22	13.40	11.35	1.01	13.25	51	14.10	11.13	1.16	12.15
23	14.45	11.49	0.82	17.60					
24	13.80	10.99	0.91	15.15	Mean	14.00	10.89	1.05	13.60
25	13.00	10.20	1.14	11.40					
26	14.45	11.28	1.22	11.85	P.E. mean.	±0.07	±0.06	±0.01	±0.17
27	14.30	11.15	1.05	13.60					
28	14.60	11.61	1.12	13.05	P.E. sing.	±0.5	±0.4	±0.09	±1.3
29	14.55	11.40	0.87	16.70					

case fifty-one), and Σd^2 is the sum of the squares of the deviations of each measurement from the mean. For example, in the column under brix, table I, "d" is the deviation of 12.80 from 14.00, etc.

The probable error of a single sample and the probable error of the mean are connected in the following manner: P.E. mean $= \frac{\text{P.E. sing.}}{\sqrt{n}}$, so that after a value for P.E. sing. has been found, the

value of P.E. mean for any desired number of fruits may be calculated by substituting this number for "n" in the formula. Thus if P.E. sing. has been found to be 0.5, P.E. mean for a sample of twenty-five fruits is $\frac{0.5}{\sqrt{25}}=0.1$.

The values in table II, giving the odds, may be utilized under the two following conditions. In the first place, it may be used in connection with the analytical results obtained from a single lot of fruit to estimate the degree of assurance that an accuracy between certain limits has been attained. For example, the average sugar content (in table I) was 10.89. If a second sample of fifty-one fruits had been taken at the same time and under the same conditions, we would probably not have obtained exactly this value.

TABLE II*

TABLE OF ODDS

Coefficient	Odds	Coefficient	Odds
1.0.....	1.00 to 1	3.4.....	44.87 to 1
1.5.....	2.21 to 1	3.6.....	64.79 to 1
2.0.....	4.64 to 1	3.8.....	95.15 to 1
2.5.....	9.89 to 1	4.0.....	142.26 to 1
2.8.....	15.95 to 1	4.2.....	215.92 to 1
3.0.....	22.26 to 1	4.4.....	332.33 to 1
3.2.....	31.36 to 1	4.6.....	519.83 to 1

* The values in this table were selected from a table by PEARL and MINER (6). Original article should be consulted for a complete list of values.

But the P.E. mean, ±0.06, indicates that the chances are even (1 to 1) that the value found would have been between 10.95 and 10.83. In addition to this information, table II shows that the chances are 9.89 to 1 that the value would have been between 10.89 plus (2.5×0.06) and 10.89 minus (2.5×0.06), that is, between 11.04 and 10.74.

Considering the probable error of a single sample in connection with table II, the P.E. sing. was found to be 0.4. This means that if one more fruit had been taken, the chances are even that its value would have been between 10.89+0.4, and 10.89−0.4. In other words, half the fruits in table I should have sugar values between 11.29 and 10.49, and half should be outside these limits. Table I shows that twenty-four oranges are within these limits and

twenty-seven outside. Table II indicates further that the chances are 4.64 to 1 that no single sample would deviate from 10.89 by as much or more than 2.0 times 0.4: that is, of the fifty-one fruits in table I, about nine should be outside the limits 11.69 to 10.09, and forty-two should be within them. A count shows that in this case five are outside and forty-six within.

In the second place, table II may be applied in an entirely different case, namely, when comparing the analytical results from two different lots of fruit in order to estimate the degree of assurance that the difference shown between them is significant. For example, in table VII it is shown that the refractive index of the juice of the Eureka strain of lemons was 44.6 ± 0.2, while that of the Shade Tree strain was 45.7 ± 0.3. The difference is 1.1. What are the chances that this difference is significant and not due merely to a sampling error? This calculation is made from the following formula: $\frac{\text{difference}}{\text{P.E. of difference}} = \frac{1.1}{\sqrt{(0.2)^2+(0.3)^2}} = \frac{1.1}{0.36} = 3.0$. The figure 3.0 is here termed the coefficient of odds, and its value is sought in column 1 in table II, from which it appears that the odds are about 22 to 1 (judging from these data, at least) that the juice of lemons from the Shade Tree strain is higher with respect to refractive index. Table II applies only in those cases in which the difference between two results may be expected to occur in either direction. For a table showing odds when it is known that the difference between two results will be in one direction only, see Wood (11, p. 26).

Formulas for calculating number of fruits for sample

Two general sets of conditions may be recognized under which samples are collected for analysis: (1) When samples are taken from each of two or more different lots of fruit, with the object of later comparing them, to determine whether the differences between them are significant, and what the odds are that this is so. (2) When a sample is taken from a single lot of fruit for the purpose of obtaining a figure that will represent the composition of that lot, and to attain a certain assurance that this figure is correct within certain desired limits.

HAYNES and JUDD (3) have studied the requirements under the first condition. They proposed the following formula for use in calculating the number of individuals to include in a sample in order that a certain difference between two averages may be considered significant: $N = 2\left(\frac{3 \times p}{5}\right)^2$. N is the "number of samples which must be taken in order that there may be a probability of 0.957[2] that a 5 per cent difference is significant"; 3 is the coefficient in the "table of odds" (table II), and thus is equivalent to odds of 22 to 1; "p" is the probable error of a single sample and must be determined experimentally (in this case by analyzing individual fruits).

Other values than 3 and 5 may be assumed to meet the conditions of the experiment; therefore, in order to make comparisons with what is to follow, it is desired to express the preceding formula in general terms as follows: $N = 2\left(\frac{\text{coefficient of odds} \times \text{P.E. sing.}}{\text{difference}}\right)^2$ (*formula 1*). To illustrate the use of this formula, data may be taken from HAYNES and JUDD'S paper. Working with apples, they found the mean titration value to be 10.20 with a P.E. sing. of 0.78, and the latter is thus 7.7 per cent of the mean. To get an assurance of 30 to 1 that a 5 per cent difference is significant: $N = 2\left(\frac{3.2 \times 7.7}{5}\right)^2 = 49$ apples.

The problem under the second condition may now be considered. We wish a general formula that will connect the number in the sample with the probable error of a single fruit and with the coefficients in the "table of odds" (table II). In table I it was shown that the mean sugar content was 1089. ± 0.06. What are the chances that the "true" value is within the limits ± 0.17? The chances are found in the following way (MERRIMAN 5): $\frac{0.17}{0.06} = 2.8$, and looking up the coefficient 2.8 in table II, we find the chances are about 16 to 1 that the error in 10.89 is not more than ± 0.17.

[2] The expression 0.957 may be thought of as indicating a probability of 957 out of 1000, which represents a ratio of 957 to 43, or about 22 to 1.

This relation may now be expressed in general terms by putting "deviation" for ±0.17, where it is to be the deviation above or below the mean, which we wish to use as a limit for accuracy; then putting "P.E. mean" for 0.06, and "coefficient of odds" for 2.8, we have: $\frac{\text{deviation}}{\text{P.E. mean}} = \text{coefficient of odds}$, but $\text{P.E. mean} = \frac{\text{P.E. sing.}}{\sqrt{N}}$ (WOOD 11), and substituting this value, the equation becomes $\frac{\text{deviation}}{\frac{\text{P.E. sing.}}{\sqrt{N}}} = \text{coefficient of odds}$, from which

$$N = \left(\frac{\text{coefficient of odds} \times \text{P.E. sing.}}{\text{deviation}}\right)^2 \text{ (\textit{formula 2}).}$$

In illustration of the use of this formula, table VI shows that fifty grapefruits from tree no. 1 had an average brix of 13.15 and the P.E. sing. was 0.35. What number of fruits are required to give odds of 10 to 1 that the brix of that number will be correct to ±0.15? Table II shows that for odds of 10 to 1, the coefficient of odds is 2.5, therefore $N = \left(\frac{2.5 \times 0.35}{0.15}\right)^2 =$ thirty-four grapefruits. No account is taken of errors in the method of analysis, since in the present case analytical errors are small as compared with the variability of the individual fruits with respect to the constituent. If it is desired to take analytical errors into account also, see WAYNICK (10) and ROBINSON and LLOYD (7).

Comparison of formulas

Although formulas 1 and 2 appear to be very similar, the first in fact giving values just double those of the second, certain essential differences should be pointed out. Formula 1 applies when *two different lots* are being compared, in which case the significance of the difference between them is affected by the sampling error of each lot. Formula 2 applies to the analytical results of a *single lot* only, its own error being the only one involved. Such a condition arises when an analysis is made for the purpose of reporting the composition of a product with respect to a certain constituent, or when an analysis is made to determine whether a constituent has reached a certain required value.

Accuracy of formulas

In the preceding paragraphs it was found that the use of formulas 1 and 2 gave forty-nine fruits as the required number in one illustrative case, and thirty-four as the required number under the other set of conditions. We should not be justified, however, in concluding from this test that forty-seven would be too few in the first case, and thirty-six would be more than enough in the second. With either formula it is seen that the number N depends for its value upon the value of the probable error of a single sample, and therefore it becomes necessary to inquire how variable this value is, and what effect changes in its value have upon N.

TABLE III

DIFFERENT VALUES OBTAINABLE FROM SAME LOT OF FRUIT

CALCULATIONS AFTER THE FOLLOWING NUMBER OF FRUITS ANALYZED	TAKEN IN ORDER OF ANALYSIS		TAKEN IN ORDER REARRANGED BY LOT			
			First rearrangement		Second rearrangement	
	P.E. sing. found	No. of fruits required	P.E. sing. found	No. of fruits required	P.E. sing. found	No. of fruits required
10	1.1	22	1.4	36	1.0	18
15	1.1	22	1.4	36	1.2	26
20	1.0	18	1.4	36	1.2	26
25	1.1	22	1.4	36	1.2	26
30	1.1	22	1.4	36	1.4	36
35	1.1	22	1.4	36	1.4	36
40	1.1	22	1.4	36	1.3	31
45	1.2	26	1.3	31	1.3	31
51	1.3	31	1.3	31	1.3	31

It is instructive to note what values would have been obtained if the value of P.E. sing. had been taken, not after fifty-one fruits had been analyzed, but after the analysis of say ten fruits, or after fifteen, or twenty-five. The different values for P.E. sing. and N that were obtainable in this manner calculated from formula 1 are shown in table III. It is thus found the P.E. sing. varied from 1.0 to 1.3, which values, substituted in the formula, caused the value of N to vary from 18 to 31. Formula 2 would likewise have given variable values, but the actual figures would have been one-half as large.

The fruits in table I were analyzed in the order of size, number one being the largest. It may be urged that therefore we do not

have a true random sample, or that there is a correlation between size and composition. The correlation coefficient between size and the soluble-solids-acid ratio, however, was calculated by the method recommended by TOLLEY (**9**), and was found to be 0.158, with a probable error of 0.092, which does not indicate any significant correlation.

In order to partially eliminate the size of the fruit as a factor, the order in table I was rearranged by lot. With the new order,

TABLE IV

RESULTS OF CALCULATIONS OF PROBABLE ERROR BASED ON ANALYSIS OF GROUPS OF TEN FRUITS EACH

GROUPS OF 10 FRUITS EACH	SOLIDS-ACID RATIO	
	P.E. sing.	No. required for desired assurance
Group 1	1.0	18
2	0.9	15
3	1.3	31
4	1.0	18
5	1.6	47
6	1.4	36
7	1.3	31
8	1.3	31
9	1.3	31
10	0.5	5
11	1.8	59
12	1.3	31
13	1.1	22
14	0.9	15
15	1.2	26

the values of P.E. sing. and N were calculated after ten fruits were analyzed, after fifteen, etc. The results are shown in table III. P.E. sing. was found to vary from 1.3 to 1.4, causing N to vary from 31 to 36. Another rearrangement by lot is shown in the last two columns of table III. Values of P.E. sing. vary from 1.0 to 1.4, causing N to change from 18 to 36. In both these cases, values by formula 2 would also have been variable, but of course would have been just half as large numerically.

Use of small numbers to calculate probable error of single fruit

It may be inquired what the P.E. sing. would have been for different lots of ten fruits each. Groups of ten each were selected

by lot and the values of P.E. sing. and N calculated. Strictly speaking, when the number involved is small, say ten, the formula for P.E. only gives approximate results (BRUNT 1). The value of P.E. sing. for the ratio is thus shown to vary from 0.5 in group 10 to 1.8 in group 11, causing a change in N from 5 to 59 (table IV). One trial with a small number of fruits would not be adequate for the determination of the value of P.E. sing. and of N, at least with such variable material as oranges.

Probable error of a probable error

The preceding discussion indicates that variable values were found for N, depending on the value found for P.E. sing. To obtain an idea of the variability of P.E. sing. and of N in the manner described (that is, by obtaining the results given by several different groups containing different numbers) is tedious and unsatisfactory. A more convenient method of judging the accuracy of P.E. sing. and N is desired. It is plain that the probable error calculated from the analysis of fifty fruits is more representative of the lot than that calculated from ten fruits. The relation of the error in the probable error to the number of fruits analyzed is given by the expression (BRUNT 1, p. 57): Probable error of P.E. sing. = P.E. sing. $\times \frac{0.4769}{\sqrt{n-1}}$ (*formula 3*). Thus if 1.3 is the P.E. sing. for the soluble-solids-acid ratio (table I), then the probable error of $1.3 = 1.3 \times \frac{0.4769}{\sqrt{51-1}} = 0.09$, or about 0.1. In other words, the "true" value of P.E. sing. is probably between 1.2 and 1.4. We may obtain an estimate of the limits of N by substituting 1.2 and 1.4 successively in the formulas; in this case N is found to be 26 or 36 for formula 1, and 13 or 18 for formula 2.

Ordinarily it will be sufficient to consider the probable limits of the value of N by approximations made by the use of formula 3 in the manner indicated. If it is found desirable to do so, however, a formula may be used for the correction. If we rearrange formula 1 to read: $N = 2\left(\frac{\text{coefficient}}{\text{difference}}\right)^2 (\text{P.E. sing.})^2$, and apply the method described by GOODWIN (2), we find that deviation produced in the

value of N by an error in the value of P.E. sing. is as follows:

$$\text{Deviation in } N = \frac{d\left[2\left(\frac{\text{coefficient}}{\text{difference}}\right)^2 (\text{P.E. sing.})^2\right]}{d(\text{P.E. sing.})} \times \text{error in P.E. sing.,} = 4\left(\frac{\text{coefficient}}{\text{difference}}\right)^2 \times \text{P.E. sing.} \times \text{error in P.E. sing. } (\textit{formula 4}).$$

To apply this formula to a particular case, we find from table III that the P.E. sing. for fifteen fruits was 1.1; the error in 1.1 is found by substituting in formula 3 to be $1.1 \times \frac{0.4769}{\sqrt{15-1}} = 0.14$. If we wish odds of 22 to 1 for a difference of 1.0 in ratio, we obtain, by substitution in formula 4: Deviation in $N = 4 \times \left(\frac{3.0}{1.0}\right)^2 \times 1.1 \times 0.14 =$ six fruits, therefore the corresponding value, 22, found in table III, is in error by six fruits, and the probable number extends from 16 to 28.

The corresponding formula for applying a correction to formula 2 is: Deviation in $N = 2 \times \left(\frac{\text{coefficient}}{\text{difference}}\right)^2 \times$ P.E. sing. $\times$ deviation in P.E. sing. (*formula 5*).

Data on other lots of oranges

The discussion thus far has related to the data from only one lot of oranges from a single tree. Fruits from four other trees were obtained and analyzed in the same manner. The number of fruits used was small, but some idea of the accuracy of the probable errors can be obtained by applying formula 3. The data are shown in table V, and serve to indicate values of P.E. sing. that may be expected in dealing with different lots of oranges.

Data on grapefruit

Fifty fruits were taken at random from a grapefruit tree in one grove, and a corresponding number from another tree located in another grove. The fruits were analyzed individually and the mean and P.E. sing. determined. To save space, the complete analyses are not given, but the results are summarized in table VI. From this table it is seen that P.E. sing. of the fruit from the two lots is

approximately the same with respect to brix and sugar, but P.E. sing. for acid and for ratio is considerably different in the two lots.

Data on lemons

That different lots of fruit show different values for P.E. sing. is also apparent from the analysis of individual lemons. In table VII will be found the results of the analysis of thirty lemons from two different lemon trees, each tree representing a different strain

TABLE V

SHOWING DIFFERENT VALUES OF P.E. SING. WITH DIFFERENT LOTS OF ORANGES

TREE NO.	NO. OF FRUITS IN SAMPLE	DEGREES BRIX		PERCENTAGE ACID		$\frac{\text{Sol. sol.}}{\text{acid}}$ ratio	
		Mean	P.E. sing.	Mean	P.E. sing.	Mean	P.E. sing.
2	12	13.70	0.5	0.87	0.07	15.9	1.1
3	13	15.00	0.5	0.86	0.04	17.4	0.8
4	12	11.80	0.4	0.79	0.08	15.2	1.3
5	9	12.45	0.3	1.46	0.10	8.6	0.7

TABLE VI

COMPARISON OF COMPOSITION OF FRUIT FROM TWO GRAPEFRUIT TREES

TREE NO.	TOTAL NO. OF FRUITS	BRIX		PERCENTAGE SUGAR		PERCENTAGE ACID		SOLIDS-ACID RATIO	
		Mean	P.E. sing.	Mean	P.E. sing.	Mean	P.E. sing.	Mean	P.E. sing.
1	50	13.15	0.35	8.16	0.27	2.29	0.01	5.8	0.2
2	50	12.30	0.35	7.89	0.29	1.65	0.09	7.5	0.4

of the Eureka variety. While too much reliance cannot be placed on the values obtained by analyzing fifteen fruits, it is seen from the table that the two lots of fruit probably have different values of P.E. sing. with respect to three of the characters of which analytical results were obtained.

Further precautions regarding use of formulas

Two further precautions may now be added regarding the use of the formulas. When the value of P.E. sing. has been found for one tree or lot of fruit, it must not be assumed that another tree

or lot will have the same value (compare acidity of two grapefruit trees, table VI). When two trees or lots of fruit are found to have the same value for P.E. sing with respect to one constituent, it must not be assumed that they agree also with respect to other constituents (compare trees no. 1 and no. 2, table VI, with respect to brix and ratio).

TABLE VII

VARIABILITY IN COMPOSITION OF INDIVIDUAL LEMONS, EUREKA VARIETY

EUREKA STRAIN*					SHADE TREE STRAIN*				
Lemon no.	Sp. Gr. of fruit	Percentage rind	Refrac. index of juice	Acidity of juice cc. NaOH	Lemon no.	Sp. Gr. of fruit	Percentage rind	Refrac. index of juice	Acidity of juice cc. NaOH
1.....	0.92	40	42.9	27.2	1.....	0.96	41	42.1	24.3
2.....	0.96	40	44.2	28.5	2.....	0.94	59	45.4	24.4
3.....	0.94	50	44.2	28.8	3.....	0.96	50	45.8	24.9
4.....	0.96	49	44.8	29.7	4.....	0.98	36	44.9	26.8
5.....	0.95	49	45.2	27.9	5.....	0.98	47	46.8	21.8
6.....	0.94	46	44.6	30.7	6.....	0.95	62	47.0	24.0
7.....	0.96	48	45.6	30.5	7.....	0.95	56	45.8	24.5
8.....	0.95	35	44.0	28.7	8.....	0.96	54	45.6	22.9
9.....	0.94	46	43.6	28.1	9.....	0.98	47	45.4	22.0
10.....	0.96	50	46.3	25.1	10.....	0.99	54	47.6	20.8
11.....	0.95	50	45.0	27.8	11.....	0.96	48	45.7	26.6
12.....	0.95	48	45.6	28.0	12.....	0.98	39	45.7	26.3
13.....	0.96	57	43.8	29.3	13.....	0.98	51	46.2	22.6
14.....	0.96	39	43.9	28.0	14.....	0.98	54	.....	20.9
15.....	0.97	49	45.4	28.7	15.....	0.97	59	.....	22.0
Mean....	0.95	46	44.6	28.5	Mean.....	0.97	50	45.7	23.7
P.E. mean	±0.002	±1.0	±0.2	±0.2	P.E. mean.	±0.003	±1.3	±0.3	±0.4
P.E. sing..	±0.007	±4.0	±0.6	±0.9	P.E. sing..	±0.010	±5.0	±0.9	±1.3

* Strains described by SHAMEL, SCOTT, and POMEROY (8).

Comparison of standard formula with Peter's formula for calculating probable error of single observation

Two general methods for calculating the value of P.E. sing. are as follows:

STANDARD FORMULA

$$\text{P.E. sing.} = \pm 0.6745 \sqrt{\frac{\Sigma d^2}{n-1}}$$

PETER'S FORMULA

$$\text{P.E. sing.} = \pm 0.8453 \frac{\Sigma d}{\sqrt{n(n-1)}}$$

Thus, to use the standard formula, the sum of the squares of the deviations must be found, while with Peter's formula only the

sum of the deviations (taken without regard to sign) is needed. Inasmuch as the latter method is more convenient, it seemed profitable to show the difference in the value of P.E. sing. given by the two methods. In table VIII are shown the comparative values found.[3] It is seen that the difference in the value of P.E. sing. by the two methods is at least not more than is shown between two groups of even the same lot of fruit. Hence no large error would have been introduced by the use of the more convenient Peter's formula.

TABLE VIII

COMPARISON OF STANDARD FORMULA WITH PETER'S FORMULA FOR CALCULATING PROBABLE ERROR OF SINGLE OBSERVATION

No. of fruits in sample	P.E. sing. observation: Solids-acid ratio		No. of fruits in sample	P.E. sing. observation: Percentage sugar	
	Standard formula	Peter's formula		Standard formula	Peter's formula
10	1.09	1.08	10	0.39	0.40
15	1.01	.99	15	0.34	0.34
25	1.09	1.10	20	0.44	0.42
30	1.10	1.13	25	0.42	0.43
40	1.09	1.02	35	0.40	0.40
45	1.20	1.22	45	0.40	0.40
51	1.26	1.29	51	0.39	0.38

Summary

1. Formulas are given, for use under two different conditions of sampling, to determine the number of fruits required in a sample in order to give a desired assurance that a certain accuracy has been attained.

2. Approximately 250 fruits of oranges, lemons, and grapefruit were analyzed individually, and the probable errors calculated. The data so obtained were applied to the formulas, and numerical examples worked out to illustrate their use.

3. It is shown that the values given by the formulas are only approximately correct. The sources of error are discussed, and formulas given by which the amount of this inaccuracy may be estimated under different conditions.

[3] Computations are made much easier by the use of tables given by MELLOR (4).

4. Analyses of fruits taken from different orange, lemon, and grapefruit trees are given, showing the variability of the fruits of different trees with respect to brix of juice, percentage of sugar, acidity, etc., and the values of the probable errors that such variability produced.

The writer wishes to express appreciation to Mr. E. M. CHACE and Mr. C. G. CHURCH for cooperation in obtaining the analytical data and for criticism of the manuscript.

UNITED STATES DEPARTMENT OF AGRICULTURE
LABORATORY OF FRUIT AND VEGETABLE CHEMISTRY
LOS ANGELES, CAL.

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UREDINALES COLLECTED BY R. THAXTER AND J. B. RORER IN TRINIDAD[1]

J. C. ARTHUR

(WITH FOUR FIGURES)

During a visit to Trinidad extending from December 19, 1912, to May 16, 1913, made by Dr. ROLAND THAXTER of Harvard University for the purpose of collecting insects and fungi, especially Pyrenomycetes, a number of Uredinales were secured, chiefly in the latter half of the period. The island of Tobago was also visited for a day in May. This island lies about thirty miles northeast of Trinidad, and geographically is to be considered with it in a study of the flora. The two islands lie close to the northern coast of South America, and in their faunal and floral relations belong to that continent.

The Uredinales, consisting of about forty-five numbers, including two from Tobago, were recently submitted to the writer for study by THAXTER. They have been found to represent thirty-seven species of rusts, and to fall into the unusually large percentage of fourteen genera. Two of the species appear to be undescribed, and one of these seems sufficiently distinctive to be the type of a new genus.

Little is known of the rusts of Trinidad, and the independent publication of this interesting list of species appears to be worth while. A few numbers have been added, which were collected in 1915 and 1916 and sent to the writer by Mr. JAMES B. RORER, Mycologist to the Board of Agriculture of Trinidad from 1909 to 1918, and earlier assistant in botany at Harvard University. He sent four numbers, which show the same high percentage of species and genera as those from THAXTER, there being four species, one of them a most interesting undescribed form, and three genera. All of the species and two of the genera are unrepresented in the THAXTER collection.

[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station.

In the following list nos. 6, 8, 14, 18, 19, 20, 30–36, thirteen in all (that is, 30 per cent) are short cycle species, while the others drop into various categories of long cycle species, five of them, nos. 1, 17, 23, 26, and 28, being actually or potentially heteroecious. Altogether the list embraces forty-three species of rusts, under sixteen genera, an excellent beginning to the study of the Uredinales of Trinidad and its adjacent islands.

For those species which have received treatment in the seventh volume of the *North American Flora* reference is made to the page where the description and synonomy occur; for the other species reference is given to the place of the original description. Thanks are especially due to Dr. THAXTER and Mr. RORER for the drawing and photographs included in this paper, illustrating two of the new species.

1. COLEOSPORIUM IPOMOEAE (Schw.) Burr. N.A.F. 87.—On *Ipomoea glabra* (Aubl.) Choisy, St. Anns Valley, February 1913, II, *Thaxter* 4.

Somewhat common throughout the West Indies in its uredinial stage.

2. PHAKOPSORA CROTONIS (Cooke) Arth. N.A.F. 104.—On *Croton gossypifolium* Vahl, La Seiva Valley, May 5, 1913, II, *Thaxter* 43.

3. PHAKOPSORA VITIS (Thüm.) Sydow, N.A.F. 102 (*Uredo Vitis* Thüm.).—On cultivated grape (*Vitis* sp.), Port of Spain, October 1916, II, *Rorer*.

4. CEROTELIUM FICI (Cast.) Arth. Bull. Torr. Bot. Club **44**: 509. 1917.—On *Ficus radula* Willd., Maraval Valley, May 14, 1913, II, *Thaxter*.

5. **Cerotelium minutum,** sp. nov.

II. Uredinia hypophyllous, more or less grouped on discolored spots, hidden in the pubescence of the host, small, 0.1–0.3 mm. in diameter, soon naked, pulverulent, pale yellow becoming colorless; peridium and paraphyses wanting; urediniospores obovoid or globoid, small, 14–16×17–23 μ; wall pale yellow or colorless, thin, 1 μ or less, sparingly and prominently echinulate, the pores obscure.

III. Telia hypophyllous, hidden by the pubescence, minute, 0.03–0.08 mm. in diameter, erumpent, forming columns about

30–50μ high, fuscous; teliospores catenulate, adhering laterally to form a cylindric column, ellipsoid or cuboid, 12–14×13–16μ; wall pale cinnamon-brown, uniformly thin, 1 μ or less, smooth.

On an undetermined Bignoniaceous vine, La Seiva Valley, April 1913, II, iii, *Thaxter* 38.

The host has large trumpet-shaped flowers and large, ovate, pointed leaves, pale with dense pubescence beneath. The rust is unusually minute. The presence of telia was pointed out by THAXTER, who supplied a microscope slide with sections showing the telial structure.

Maravalia, gen. nov.

Cycle of development includes telia, and possibly pycnia.

Telia subepidermal, erumpent, somewhat indefinite. Teliospores free, one-celled, with apical germination, pedicellate; wall colorless, thin, smooth.

6. **Maravalia pallida** Arthur and Thaxter, sp. nov.

O. Pycnia unknown, probably not formed.

III. Telia hypophyllous, numerous in circular areas 5–10 mm. across on somewhat larger yellowish spots, at first pulvinate, roundish, 0.2–0.4 mm. across, becoming larger, elongate and branched, somewhat confluent, early naked, yellowish becoming white, velvety by germination, ruptured epidermis erect or somewhat overarching; teliospores elongate-oblong, clavate-oblong, or cylindroid, 13–22×58–67μ, rounded at both ends or narrowed below, germinating upon maturity; wall colorless, uniformly very thin, about 0.5μ, smooth; pedicel slender, 8–10μ in diameter, 20–35μ long, colorless.

On *Pithecolobium latifolium* (L.) Benth. (*Zygia latifolia* St. Hil.), Maraval Valley, April 1913, *Thaxter*.

The genus differs from *Chaconia*, established by JUEL for a white rust on *Pithecolobium divaricatum* (Bong.) Benth., from Paraguay, by the mode of origin of the spores. Both are short cycle species; in *Chaconia* the spores are sessile and clustered on a large basal cell, while in *Maravalia* they are pedicelled and arise directly from the hymenial layer of hyphae in the same manner as is usual in the large majority of rusts (fig. 1). The genus is apparently a short cycle condition of the genus *Spirechina*. *M. pallida* much resembles in gross appearance *S. epiphylla*, a Texan rust on *Rubus*. In its teliospores it also much resembles *S. epiphylla*, and there is even a closer resemblance to those of *S. Pittieriana*, another *Rubus* rust from Costa Rica. Figs. 1 and 2 well illustrate the structure and habit of the rust.

7. **Milesia Blechni** (Sydow), comb. nov. (*Melampsorella Blechni* Sydow, Ann. Myc. **1**:537. 1903; *Uredo Blechni* Diet. and Neg., Engler's Jahrb. **22**:358. 1896).—On *Lygodium polymorphum* (Cav.) H.B.K., St. Anns Valley, February 20, 1913, II, *Thaxter* 45.

A common rust in Europe, and also known from Chile. The host is new for the species. Special interest attaches to the group of fern rusts to which this one belongs, as probably representing the most primitive characters of Melampsoraceae, and possibly of all the rusts as well.

8. CIONOTHRIX PRAELONGA (Wint.) Arth. N.A.F. 124.—On *Eupatorium odoratum* L., La Seiva Valley, April 1913, *Thaxter* 41.

A common short cycle rust occuring throughout tropical America on various species of *Eupatorium*.

9. RAVENELIA INDIGOFERAE Tranz. N.A.F. 144.—On *Indigo-fera suffruticosa* Mill. (*I. Anil* L.), Roxborough, Tobago, May 8–9, 1913, II, *Thaxter* 25.

10. DICHEIRINIA BINATA (Berk.) Arth. N.A.F. 147 (*Uredo Cabreriana* Kern and Kellerm.).—On *Erythrina umbrosa* H.B.K., Palmiste, San Fernando, October 24, 1916, II, *Rorer*.

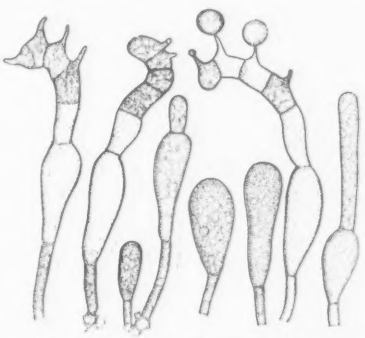

FIG. 1.—Young and mature teliospores of *Maravalia pallida*, the latter germinating.

"The host is called Anauca Immortal, and is used to shade cacao. The rust attacks not only the leaf blades, but the midribs and petioles, sometimes causing distortion," as writes Mr. RORER. It is not uncommon in other West Indian islands and in Central America.

11. CTENODERMA CRISTATUM (Speg.) Sydow, Ann. Myc. **17**:103. 1919 (*Uromyces Cupaniae* Arth. and Johnst.).—On *Cupania americana* L., Maraval Valley, April 1913, III, *Thaxter* 40.

12. DESMELLA GYMNOGRAMMES (P. Henn.) Sydow, Ann. Myc. **16**:242. 1918.—On *Adiantum latifolium* Lam., Maraval Valley, March 1913, II, *Thaxter* 44; *Cyclopeltis semicordata* (Sw.) J. Sm., Maraval Valley, 1913, II, *Thaxter* 46.

This is a common fern rust in its uredinial stage throughout tropical America on many genera of ferns. It has been reported from Porto Rico on the first named host, but the second host is a new record. The teliospores have recently come to light on a collection from Brazil of *Lygodium polymorphum* (Cav.) H.B.K. in the National Herbarium. I am indebted to Mr. W. R. MAXON for the opportunity of studying this interesting material, and also for his kindness in determining the ferns mentioned in this paper. The genus *Desmella* possesses by far the most primitive characters of any rusts known in the family Aecidiaceae (Pucciniaceae), and consequently its members have unusual interest for the student of Uredinalean evolution.

FIG. 2.—Leaf of *Pithecolobium latifolium* showing groups of sori of *Maravalia pallida*, nearly natural size.

13. UROMYCES COLUMBIANUS Mayor, Mém. Soc. Neuch. **5**:467. 1913.—On *Melanthera aspera* (Jacq.) Steud., Manzanilla Beach, 1913, II, *Thaxter* 33; Maraval Valley, 1913, i. II. *Thaxter* 34.

14. UROMYCES JAMAICENSIS Vesterg. Ark. Bot. Stockh. 4^{15}:33. 1905.—On *Bauhinia Pauletia* Pers., St. Anns Valley, March-April 1913, *Thaxter* 20a; La Seiva Valley, April-May 1913, *Thaxter* 20b.

A short cycle species known heretofore only from the North American West Indies and Mexico.

15. UROMYCES WULFFIAE-STENOGLOSSAE Diet. Ann. Myc. **6**:96. 1908.—On *Wulffia baccata* (L.f.) Kuntze, St. Anns Valley, 1913, III, *Thaxter* 35; La Seiva Valley, 1913, III, *Thaxter* 36.

16. PUCCINIA ACNISTI Arth. N.A.F. 471.—On *Acnistus arborescens* Schlecht., Maraval Valley, 1913, I, *Thaxter* 23.

17. PUCCINIA ANTIOQUINENSIS Mayor, N.A.F. 347.—On *Cyperus diffusus* Vahl, Sangre Grande, April 1913, III, *Thaxter* 51.

18. PUCCINIA ARECHAVALETAE Speg. Ann. Soc. Ci. Arg. **12**:67. 1881.—On *Urvillea Seriana* (L.) H.B.K., St. Claire, Port of Spain, February–April 1913, *Thaxter* 28.

19. **Puccinia corticola** Arthur and Rorer, sp. nov.

O. Pycnia amphigenous, rather few, seen in groups of 2 or 3, inconspicuous, subepidermal, flask-shaped, 74–96μ broad by 110–115μ high; ostiolar filaments agglutinated.

III. Telia amphigenous and caulicolous, on leaves and young twigs, few, scattered, confluent in groups of 6–8 or more, on leaf blades rounded, on midribs and stems oblong or lenticular, 0.2–1.3 mm. long, on trunk and branches much larger, rather early naked, dark brown, pulverulent, ruptured epidermis evident; teliospores ellipsoid, 19–24×30–42μ, rounded above and below, slightly or not constricted at septum; wall cinnamon or chestnut brown, 2–2.5μ thick, slightly thicker at apex, coarsely and sparsely verrucose, the markings more pronounced above, pedicel colorless, fragile.

On *Cordia Gerascanthus* L. (Ehretiaceae), Arima Forest Reserve, December 1915, *Rorer*.

The host, Mr. RORER writes, is "a timber tree, which yields a wood known as Cypre. The disease is of economic importance here, as it not only attacks the leaves and young twigs, but the older branches as well, and causes large lesions on the main trunk of the tree." Figs. 3 and 4 show the injury to five year old trees. This interesting short cycle species is the only rust in *Puccinia* known to attack the trunks and larger branches of trees, although some species of *Gymnosporangium* show such behavior.

20. PUCCINIA EUPATORII DIET. Hedwigia **36**:32. 1897.—On *Eupatorium iresinoides* H.B.K., Diego Martin, 1913, *Thaxter* 32.

21. PUCCINIA GOUANIAE Holway, Ann. Myc. **3**:21. 1905.—On *Gouania polygama* (Jacq.) Urban, Maraval Valley, March 1913, II, *Thaxter* 30a; Roxborough, Tobago, May 8–9, 1913, II, iii, *Thaxter* 30b.

22. PUCCINIA HELICONIAE (Diet.) Arth. Bull. Torr. Bot. Club **45**:144. 1918.—On *Bihai Psittacorum* (L.) Kuntze (*Heliconia Psittacorum* L.), La Seiva Valley, April 1913, II, *Thaxter* 11.

23. **Puccinia** (?) **ignava** (Arth.), comb. nov. (*Uredo ignava* Arth.), N.A.F. 341.—On *Dendrocalamus giganteus* Munro (?), Maraval Valley, March 1913, II, *Thaxter* 49.

FIG. 3.—Base of trunk of a five-year-old tree of *Cordia Gerascanthus*, showing severe injury caused by *Puccinia corticola*.

The host is an introduced bamboo, and one not before recorded for the rust. The name is transferred to *Puccinia* for convenience of listing. There

is large probability that when the teliospores are found they will justify this change.

FIG. 4.—Trunk of five-year-old tree of *Cordia Gerascanthus*, showing lesions made by *Puccinia corticola*.

24. PUCCINIA INVAGINATA Arth. and Johnston, Mem. Torr. Club **17**: 146. 1918.—On *Gouania polygama* (Jacq.) Urban, St. Anns Valley, May 3, 1913, II, III, *Thaxter* 29.

Heretofore known from the North American West Indies and Guatemala, but not from South America.

25. PUCCINIA LEONOTIDIS (P. Henn.) Arth. N.A.F. 407.—On *Leonotis nepetaefolia* (L.) R. Br., Maraval Valley, March 1913, II, *Thaxter* 10.

Only the uredinia of this common tropical rust are known in America.

26. PUCCINIA PURPUREA Cooke, N.A.F. 284.—On Guinea corn, *Holcus Sorghum* L., Port of Spain, September 1916, II, *Rorer*.

27. PUCCINIA RUELLIAE (Berk. and Br.) Lagerh. N.A.F. 415.—On *Blechum Brownei* Juss., La Seiva Valley, 1913, II, *Thaxter* 2; *Dianthera pectoralis* (Jacq.) Gmel. (*Justicia pectoralis* Jacq.), Port of Spain, 1913, II, *Thaxter* 3.

28. PUCCINIA SCLERIAE (Paz.) Arth. N.A.F. 349.—On *Passiflora tuberosa* Jacq., St. Anns Valley, February-March 1913, I, *Thaxter* 18; *Scleria* sp., Sangre Grande, April 1913, II, *Thaxter* 52.

Cultures establishing the relation of the alternate hosts of this species were made by Mr. H. E. THOMAS in Porto Rico in 1917 (Phytopath. **8**:163–164. 1918).

29. PUCCINIA SMILACIS Schw. N.A.F. 377.—On *Smilax cumanensis* Willd. (?), La Seiva Valley, March-April 1913, II, *Thaxter* 17.

Not before reported for the South American region.

30. PUCCINIA SPEGAZZINII DeToni, in Sacc. Syll. Fung. **7**:704. 1888.—On *Mikania* sp., Maraval Valley, April 1913, *Thaxter* 7.

31. PUCCINIA SYNEDRELLAE P. Henn. Hedwigia **37**:277. 1898.—On *Emilia sonchifolia* (L.) DC., La Seiva Valley, April-May 1913, *Thaxter* 22; *Synedrella nodiflora* (L.) Gaertn., Sangre Grande, April 1913, *Thaxter* 42.

32. PUCCINIA TRIUMFETTAE Dietel and Holway; Holway, BOT. GAZ. **24**:30. 1897.—On *Triumfetta* sp., Maraval and La Seiva Valley, April-May 1913, *Thaxter*.

Heretofore scantily represented by collections from southern Mexico and Ecuador. The collection here cited also bore *Pucciniosira pallidula* rather intimately associated on the same leaves.

33. ENDOPHYLLUM GUTTATUM (Kunze) Sydow, Ann. Myc. **17**:179. 1920 (*E. circumscriptum* Whetzel and Olive).—On *Cissus sicyoides* L., Sangre Grande, February 1913, *Thaxter* 24.

34. ENDOPHYLLUM PUMILIO (Kunze) Sydow, Ann. Myc. **17**:179. 1920. (*E. decoloratum* Whetzel and Olive.)—On *Clibadium surinamense* L., Maraval Valley, 1913, *Thaxter* 37.

35. ENDOPHYLLUM WEDELIAE (Earle) Whetzel and Olive, Amer. Jour. Bot. **4**:49. 1917.—On *Wedelia trilobata* (L.) Hitchc., Manzanilla Beach, March 1913, *Thaxter* 5b.

36. PUCCINIOSIRA PALLIDULA (Speg.) Lagerh. N.A.F. 127.—On *Triumfetta* sp. Maraval Valley, 1913, *Thaxter* 27. Also associated with *Puccinia Triumfettae* in another collection.

37. UREDO ADENOCALYMMATIS P. Henn. Hedwigia **35**:249. 1896.—On Bignoniaceae, La Seiva Valley, April 1913, *Thaxter* 39.

38. UREDO CHERIMOLIAE Lagerh. Bull. Soc. Myc. Fr. **11**:215. 1895.—On *Rollinia multiflora* Splitg., La Seiva Valley, *Thaxter* 14.

A new host for this common Anonaceous rust.

39. UREDO MANDEVILLAE Mayor, Mém. Soc. Neuch. **5**:591. 1913.—On *Mandevilla tomentosa* (Vahl) Kuntze, Aripo Savanna, April 1913, *Thaxter* 9a; La Seiva Valley, April 1913, *Thaxter* 9b.

40. UREDO PHYLLANTHI P. Henn. Hedwigia **35**:248. 1896.—On *Phyllanthus Conami* Sw. (*P. acuminatus* Vahl), Maraval Valley, March 1913, *Thaxter* 31.

The species has previously been recorded from the vicinity of Rio de Janeiro, Brazil.

41. UREDO RUBESCENS Arth. Mycologia **7**:327. 1915.—On *Dorstenia Contrajerva* L., Maraval Valley, January 1913, *Thaxter* 13.

Also known from Porto Rico and Guatemala.

42. UREDO SABICEICOLA Arth. Mycologia **7**:323. 1915.—On *Sabicea aspera* Aubl., La Seiva Valley, March-April 1913, *Thaxter* 19a, b.

Heretofore only known from Porto Rico.

43. AECIDIUM BRASILIENSE Diet. Hedwigia **36**:35. 1897.—On *Cordia* sp., Maraval Valley, 1913, *Thaxter* 15, 16.

Previously reported from southern Brazil.

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Triumfetta sp. 32, 36
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Vitis sp. 3
Wedelia trilobata 35
Wulffia baccata 15
Zygia latifolia 6

PURDUE UNIVERSITY
LAFAYETTE, IND.

CLASSIFICATION OF THE ANAEROBIC BACTERIA[1]

HILDA HEMPL HELLER

The classification of living forms should depend on an understanding of the laws of heredity as demonstrated in those forms. Preliminary classifications are made by applying the machinery of arrangement that has been worked out for other groups that are well understood, to groups of whose biological processes we know little. Preliminary classifications are necessary and are as desirable as are catalogues, and should be made to correspond to the known life processes of the organisms as nearly as possible, but they should only be offered tentatively.

The study of the biology of the anaerobic bacilli is in its early morning twilight. Today the scientific world holds two widely opposite opinions in regard to the classification of these organisms. The view held in Western Europe and in America is that the anaerobic species are many, distinguishable, and not highly variable; that held by many workers in Central Europe is that the species are practically indistinguishable, are highly variable, and may be changed one into another. It is impossible to bring these two points of view into alignment. They are not to be attributed to diverse interpretation of differences that shade into one another. They are themselves the outcome of an evolutionary process, depending on a mutation in thought, followed by the throwing up of a geographical barrier in 1914 that isolated the mutant thought and permitted it to overgrow its ancestral types of reasoning in a peculiarly favorable soil. The crux of the matter lies in the purity of the cultures studied by the classifier. It is notorious that the casual worker with anaerobic organisms knows neither how to purify them, nor how to tell when they are pure or impure. The anaerobes are not difficult to isolate when one knows how, but usually workers do not know how. Anaerobes have occasionally been isolated in pure culture since the early days of bacteriology.

[1] From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.

A number of descriptions of pure cultures exist, but it was the exceptionally studious worker who was responsible for such descriptions, and casual workers were apparently in the majority.

In 1901 GRASSBERGER and SCHATTENFROH (**18, 19**) propounded their theory of the transmutability of anaerobic species. In the period following they corroborated and extended their findings, and their work was pushed with so many publications (**16, 17, 20–23**) and with so much assertion that by 1914 it was seriously quoted in at least one well known German textbook (**39**), and the doctrine was thoroughly distributed throughout Central Europe. Under pressure of war, work is not carefully done. The casual workers found it necessary to make many anaerobic diagnoses from gas gangrene cases, which they made rapidly, and then turned to their new found collections to classify them. Many of these war workers corroborated the general findings of GRASSBERGER and SCHATTENFROH, namely, that the characters of anaerobes are highly variable, and that species among these organisms are not to be seriously studied.

CONRADI and BIELING (**4, 5**) were the most extreme in their contentions, claiming that one labile species was responsible for all gas gangrene cases. They described two cycles, one developing on carbohydrate media, the other on protein media, and claimed that immune sera identified all the strains in each cycle, but that when a strain was changed over to the other cycle culturally, it was also changed immunologically to that cycle. Such contentions struck so forcibly against all conceptions of immunity that they did not long go unchallenged. A number of workers (**12, 38**) corroborated the transmutability findings but not the immunological ones. In fact KLOSE (**31, 32**), working with highly variable impure cultures, used toxin formation to distinguish his strains. Some, such as ASCHOFF (**1**), who worked with slightly impure cultures, remained on the fence in regard to the transformations. Most assertive in their contentions that anaerobic organisms are highly variable in their reactions and are transmutable were the school of KOLLE (**34–36**), especially SCHLOSSBERGER (**43**), who suggested that the anaerobes may represent a single species. These workers sent cultures to VON WASSERMANN (**49**), who declared them indistinguishable. He

refused to submit anaerobic cultures to such drastic treatment as transatlantic shipment because of their fragility (**48**), and yet an anaerobic organism taken in muscle from a whale by NIELSEN (**40**) in 1888 responded satisfactorily to routine anaerobic technique in this laboratory in 1918, killing its guinea pig promptly and resembling exactly its original description! In the veterinary field VAN HEELSBERGEN (**24**) recently aligned himself with the German workers in human pathology.

The alteration of scientific attitude brought about by the adherence to such a theory as this is most interesting. Things become so simple under such an explanation, many technical difficulties are eliminated, immunization of animals for therapeutic purposes is made easy, and the scientific world, from the point of view of these workers, is so much the better off. For, if anaerobes may be changed one into another, why bother about isolating them? They will not stay pure. Anaerobic cultures from Central Europe that have found their way to this country are seldom pure, and frequently do not contain the type of organism for which they were named. If they contain a pathogen, two or three types may be isolated from the cultures, and these types behave consistently and do not do the queer things they were supposed to do by their first students. Central European anaerobic studies are struggling in the dark, the days of NÄGELI and BILLROTH have returned, the land of KOCH (**33**), of GHON and SACHS (**15**), and of VON HIBLER (**30**) has shifted from the careful work of older days, GHON himself (**14**) is converted to the new theories, and but a few constructive workers with abundant material have come out openly to combat them. Chief among these is ZEISSLER (**51**), a pupil of and coworker with FRAENKEL (**6–11**), who has always maintained that gas gangrene is due to various distinguishable anaerobic organisms. PFEIFFER and BESSAU (**41**) and GAEHTGENS (**13**) clearly distinguish various types. Early in the period of the war ZACHERL (**50**) and KÖVES (**37**) gave good descriptions of pure cultures of the vibrion septique type of organism.

As is to be expected, museum cultures from Central Europe are more badly mixed than any others. A collection of ten strains of anaerobes from KRAL'S in Vienna apparently did not contain a

single species for which the cultures were named. Unfortunately the anaerobe strains of some of our own institutions are but little better.

It would be difficult for the systematist employed in the study of higher plants whose major characters are well understood, whose mutations are today being scientifically studied, whose formal structure of classification was laid down many years ago and has been systematically developed, to imagine the complexity of the problem confronting one desirous of bringing order into the chaos represented not only by this war literature, but also by thirty years of anaerobic literature written before it. The time is more than ripe for some organization to enable new students to set to work with some clearness and assurance, an organization with a synopsis or index to the enormous literature that they must consult. This should give them an idea of the multiplicity of the species they will encounter, and should consider the biological factors relating to morphology, chemical behavior, and mutation as they are understood today.

Several workers have stated that anaerobic bacilli do not mutate. This is their natural reaction in denying the existence of the type of mutation that was described by the workers with impure cultures. To state that a living strain does not mutate would be to claim that it lacks one of the best recognized attributes of living matter. Obviously, it is necessary to determine where the mutations of bacteria lie, and what range of possible change they cover, before one can tell what characters are stable enough to be used for systematic purposes. The enzymes of the anaerobic bacilli are among the most highly active chemical agents known. Some of the anaerobes will be found among the most active splitters of carbohydrates, others have almost unbelievable proteolytic powers. It is to be expected that mutations will frequently be encountered in highly specialized organisms of this sort, and that these mutations will be chemical in their nature. When a mutation occurs that enables the organism to render assimilable a substance that its parent was unable to utilize, the mutant is readily detected because of the larger colony that it produces. Likewise a bacillus that loses a metabolic power forms smaller colonies than its parents. Data

pertaining to such mutations may be recorded photographically (28), and the possibilities thus afforded for the study of bacterial mutations in certain groups, notably in that of the tetanus bacillus (29), are unlimited.

The Society of American Bacteriologists (2) proposes the use of the botanical rules adopted at the Vienna Congress (44) in 1908 for the purposes of bacterial classification. In many ways the scheme formulated during a century and a half by the botanists is excellent for the purpose, although in some ways we are not ready for it. It is composed of stems and twigs and branches. When we pick up a bacterial group, we do not know whether to call it a stem or a twig or a branch, for the leaves have mostly grown on trunks. The tendency has been to work downward, to call a superficially recognized group a species and subdivide it into types, and to number the types. Why not work upward, call the numbered types species, and have more room for classification?

Bacteriologists, trained in pathological laboratories, have perhaps laid too little emphasis on the necessity of observing the laws of heredity in making classifications. It seems as though an application of these laws, with the same scale of nomenclature used by the classifiers of higher plants, might well be applied to the systematic arrangement of bacteria. Thus a tetanus strain of a pure biotype may give rise to many biotypes, as shown by colony formation. These derivative types are all typical tetanus bacilli. They represent elementary species, and are too many to catalogue, being of interest only to the student of heredity. They are no more deserving of specific names than are the commonly observed small mutations of higher plants and animals, and if named would require a trinomial nomenclature. There are some definite protein substances, however, differing radically in various tetanus bacilli, that probably are not subject to active mutation and are demonstrable by an immune reaction, agglutination. Four groups of tetanus bacilli have thus been distinguished by TULLOCH (45, 46), and four groups of vibrion septique bacilli by ROBERTSON (42). In the colon-typhoid group this reaction has long been considered specific, why not in these? To be sure, the details of the reaction

in these groups require more study, and other and better ways may be found to divide them. The following general rules will probably be found convenient for classifying bacteria.

BIOTYPE.—Strains that differ from each other in characters that are readily subject to mutation, and that breed true, may be termed biotypes. The word subspecies has so long been used in the higher groups with a geographical connotation that it will not be well to use it for subdivisions of bacterial species. The term type may then well be left as an independent unit of our vocabularies for non-specific use.

SPECIES.—Strains that behave alike in those characters that within their genus have not been found to mutate readily, may be grouped as species. The occasional derivation of one species from another is no more to be considered impossible than it is in higher groups. Bacteriologists have too long considered the species conception in higher groups as one of fixed immutable orms, which it is not. The recombination effects noted by DE VRIES (**47**), in which he showed the independent origin of three well defined types (*Oenothera nanella*, *O. eliptica*, and *O. lata*) from two others of quite widely divergent character (*O. Lamarckiana* and *O. laevifolia*) apparently cannot occur among bacteria. These recombination effects allowed the sudden appearance of groups of mutations that had occurred previously. Among bacteria, however, because of the rapidity with which vast numbers may be bred and the energy with which selection acts, several characters may be changed nearly simultaneously, and similar effects to those noted by DE VRIES may occasionally be observed, namely, the appearance of a number of new characters within a short space of time. On analogy, it would be perfectly reasonable to describe the strains that result from various changes as separate species. It is quibbling to define the word "species" so closely that no elasticity should be allowed in its application. Our knowledge is too meager and the possibilities too great to restrict closely the meanings of taxonomic words. Changes that may be considered specific may be discovered or perhaps even brought about by treatment of bacteria that is more radical than anything tried with plants, and there is no reason why

a bacteriologist who finds a new type that has originated in his own hands should not dignify it by the name of species, if he can show that such a change affects several characters.

GENUS.—Organisms that show the same general reactions on ordinary media and have the same general morphological habit may be grouped in genera. Such a scheme will compel the classification of most of the old and well recognized anaerobic species as genera, although it will unite some, such as NOVY's bacillus and *B. oedematiens*, and vibrion septique and the whale septicaemia bacillus into genera. It gives to the words species and genus approximately the same rank, in relation to mutational possibilities, as they possess among the higher forms. A vibrion septique strain can no more mutate to a sporogenes habit than can a pine tree mutate to an oak, but it can mutate in small detailed characters that may be of interest, and there are vibrion septique strains that differ in more fixed characters and that should be given specific differentiation.

A detailed plan (**27**) following these lines has already been presented. It is intended as a preliminary classification. Twenty-seven anaerobic genera have been defined, many more will have to be admitted in time, especially in the proteolytic group, and when large collections have been studied many emendations will have to be made. An organization (**26**) of these genera has been prepared, employing chemical criteria only; morphological criteria are found entirely inadequate for purposes of classification in this group. It has been thought premature to group the genera into tribes. Two subfamilies include all the anaerobic rods described in the genera.

Subfamily I. Clostridioideae.— Clostridiaceae that on meat medium produce after twenty days' incubation under oil at 37° a reaction of P_H 7.0 or a more acid reaction, the culture having been boiled after incubation. Type genus *Rivoltillus*, the vibrion septique type as described by HELLER (**25**).

Subfamily II. Putrificoideae.—Clostridiaceae that on meat medium produce after twenty days' incubation at 37° under oil a reaction of P_H 7.1 or a more alkaline reaction, the culture having been boiled after incubation. Type genus *Metchnikovillus*, the sporogenes type as defined in the description of *Bacillus sporogenes*.

described by the Medical Research Committee (3) under the name of Metchnikoff's type A. These subfamilies are united into the following family:

Clostridiaceae.—Eubacteriales that are rodlike, not spiral, that will not grow within 7 mm. of the surface of a shaft of clear tissue-free agar medium contained in a tube 12 mm. or more in diameter, incubated in air, in which they are able to grow in the depths. They may or may not possess peritrichial flagella; they may or may not form endospores. Most members of the group are characterized by their energetic catalytic action on proteins or on carbohydrates or on both of these types of substances.

UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL
SAN FRANCISCO, CAL.

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NOTES FOR STUDENTS

Forest geography of New Jersey.—HARPER subdivides New Jersey into nine forest regions, most of which form approximately parallel belts traversing the state from northeast to southwest.[1] Each of these is discussed in turn and the characteristic species noted. The most abundant tree in the state is *Pinus rigida*, but the most widely distributed tree probably is *Quercus alba*, which occurs in all of the nine forest regions; *Acer rubrum* is not far behind *Quercus alba* in this respect. Evergreen species probably make up about 40 per cent of the forests of the state.—H. C. COWLES.

Random assortment in inheritance of distinguishable homologous chromosomes.—Miss CAROTHERS,[2] from her work on Orthoptera, has already reported the occurrence of homologous chromosomes which could be identified one from the other by a size difference. Furthermore, since the form of a given homologue is constant for the individual, she has been able to demonstrate, from a study of a number of individuals of the population, that these heteromorphic homologous chromosomes (3 pairs) have a random segregation in relation to each other and to the sex chromosome. In the present paper, she actually follows these chromosomes from parent to offspring, making a cytological examination of the parents after they have been allowed to reproduce, and later an examination of the resulting progeny. Size, shape, and point of attachment of spindle fibers all seem to be practically constant heritable characters, by which the author identifies the individual chromosomes, and shows that their recombination in the progeny is according to the laws of chance. In the author's material one can say in regard to the chromosomes which enter the gametes, just as certainly as of a pair of contrasting unit characters which segregate in the F_2 generation, that this one was contributed by the father and that one by the mother. This amounts to a direct demonstration of those assumptions as to the behavior of the chromosomes in inheritance which have been necessary to account for the workings of Mendel's law. It is hoped that eventually such structural variations of the chromosomes will be correlated with the resulting somatic characters of the individual.—M. C. COULTER.

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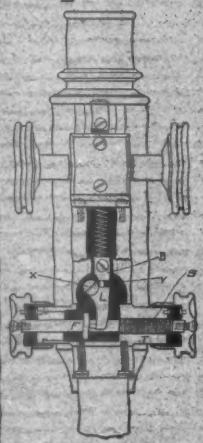

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